L1 ANSWER 1 OF 1 USPATFULL on STN

No References

2003:285090 Multivalent dengue virus vaccine.

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The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)

US 6638514 B1 20031028
APPLICATION: US 2000-535117 20000324 (9)
PRIORITY: US 2000-181724P 20000211 (60)
US 1999-126313P 19990326 (60)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

EXNAM Primary Examiner: Scheiner, Laurie; Assistant Examiner: Parkin, J. S.

CLM What is claimed is:

1. An immunogenic composition comprising two or more attenuated dengue viruses selected from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652, and a physiologically acceptable vehicle.

2. The immunogenic composition according to claim 1 which further comprises an adjuvant to enhance the immune response.

3. The immunogenic composition of claim 1, formulated in a dose of 10^2 to 10^6 PFU of attenuated virus.

4. A multivalent live attenuated dengue virus vaccine comprising any combination of dengue virus serotypes selected from the group consisting of: a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S 16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652.

5. The dengue virus vaccine of claim 4 wherein said dengue virus is produced in vertebrate cells.

6. The dengue virus vaccine of claim 5 wherein said cells are Vero cells.

7. The dengue virus vaccine of claim 4 wherein said dengue-1 virus is in the amount of 10^2 to 10^7 pfu/ml, said dengue-2 virus is in the

amount of 10^2 to 10^7 pfu, said dengue-3 virus is in the amount of 10^2 to 10^7 pfu, and said dengue-4 virus is in the amount of 10^2 to 10^7 pfu/ml.

8. The dengue virus vaccine of claim 7 wherein said vaccine is administered subcutaneously.

9. An immunogenic composition comprising two or more attenuated dengue virus chosen from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 sawn 45AZ5 PDK-27 having the ATCC accession number PTA4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-S0 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH153489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN4) virus having the sequence of DEN-4 strain 341750 PDK6 having the ATCC accession number PTA4811, and a physiologically acceptable vehicle.

10. A multivalent live attenuated dengue virus vaccine comprising any combination of dengue virus serotypes selected from the group consisting of: a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 stain 341750 PDK-6 having the ATCC accession number PTA-4811.

11. The vaccine of claim 10 wherein at least one virus os DEN-1 strain 45Az5 PDK-27 having the ATCC accessior number PTA-4810.

12. The vaccine of claim 10 wherein at least one virus is DEN-4 strain 341750 PDK-6 having the ATCC accessior number PTA-4811.

IN THE CLAIMS:

4-6, 17-39

Claims 1-3. (canceled)

WD
112 R1 Scope

Claim 4. (Original) A method for stimulating dengue virus specific immune response, which comprises administering to an individual an immunologically sufficient amount of two or more attenuated viruses chosen from the group consisting of dengue-1, dengue-2, dengue-3, and dengue-4, in a physiologically acceptable carrier.

Claim 5. (Original) The method of claim 4, wherein the attenuated virus is administered parenterally.

Claim 6. (Original) The method of claim 4, wherein the attenuated virus is administered intranasally.

Claims 7-16. (Canceled)

Claim 17. (Previously submitted) The method of claim 4, which comprises administering to an individual an immunologically sufficient amount of two or more attenuated viruses chosen from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA-4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-6 having the ATCC accession number PTA-4811, and a physiologically acceptable vehicle.

Claim 18. (Previously submitted) The method of claim 4, which comprises administering to an individual an immunologically sufficient amount of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC

accession number PTA-4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-6 having the ATCC accession number PTA-4811, and a physiologically acceptable vehicle.

Claim 19. (Previously submitted) The method of claim 4, which further comprises administering an adjuvant to enhance the immune response.

Claim 20. (Previously submitted) The method of claim 4, wherein the attenuated viruses administered are formulated in a dose of 10^2 to 10^6 PFU/ml.

Claim 21. (Previously submitted) The method of claim 4, wherein the attenuated viruses are administered subcutaneously.

Claim 22. (Previously submitted) The method of claim 4, which comprises administering to an individual an immunologically sufficient amount of two or more attenuated viruses chosen from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA-4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652, and a physiologically acceptable vehicle.

Claim 23. (Previously submitted) The method of claim 4, which comprises administering to an individual an immunologically sufficient amount of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA-4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2

strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652, and a physiologically acceptable vehicle.

Claim 24. (Previously submitted) The method of claim 4, which comprises administering to an individual an immunologically sufficient amount of two or more attenuated viruses chosen from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652, and a physiologically acceptable vehicle.

Claim 25. (Previously submitted) The method of claim 4, which comprises administering to an individual an immunologically sufficient amount of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652, and a physiologically acceptable vehicle.

Claim 26. (Previously submitted) The method of claim 4, which comprises administering to an individual an immunologically sufficient amount of two or more attenuated viruses chosen from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-

2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-6 having the ATCC accession number PTA-4811, and a physiologically acceptable vehicle.

Claim 27. (Previously submitted) The method of claim 4, which comprises administering to an individual an immunologically sufficient amount of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-6 having the ATCC accession number PTA-4811, and a physiologically acceptable vehicle.

Claim 28. (New) An immunogenic composition comprising two or more attenuated dengue viruses selected from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652, and a physiologically acceptable vehicle.

Claim 29. (New) The immunogenic composition according to claim 28 which further comprises an adjuvant to enhance the immune response.

Claim 30. (New) The immunogenic composition of claim 28, formulated in a dose of 10^2 to 10^6 PFU of attenuated virus.

Claim 31. (New) A multivalent live attenuated dengue virus vaccine comprising any combination of dengue virus serotypes selected from the group consisting of: a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S 16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652.

Claim 32. (New) The dengue virus vaccine of claim 31 wherein said dengue virus is produced in vertebrate cells.

Claim 33. (New) The dengue virus vaccine of claim 32 wherein said cells are Vero cells.

Claim 34. (New) The dengue virus vaccine of claim 31 wherein said dengue-1 virus is in the amount of 10^2 to 10^7 pfu/ml, said dengue-2 virus is in the amount of 10^2 to 10^7 pfu, said dengue-3 virus is in the amount of 10^2 to 10^7 pfu, and said dengue-4 virus is in the amount of 10^2 to 10^7 pfu/ml.

Claim 35. (New) The dengue virus vaccine of claim 34 wherein said vaccine is administered subcutaneously.

Claim 36. (New) An immunogenic composition comprising two or more attenuated dengue virus chosen from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-S0 having the ATCC accession number VR-2653, a dengue-3 (DEN-3)

virus having the sequence of DEN-3 strain CH153489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN4) virus having the sequence of DEN-4 strain 341750 PDK6 having the ATCC accession number PTA4811, and a physiologically acceptable vehicle.

STAT DP

Claim 37. (New) A multivalent live attenuated dengue virus vaccine comprising any combination of dengue virus serotypes selected from the group consisting of: a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 stain 341750 PDK-6 having the ATCC accession number PTA-4811.

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Claim 38. (New) The vaccine of claim 37 wherein at least one virus is DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA-4810.

STAT DP

Claim 39. (New) The vaccine of claim 37 wherein at least one virus is DEN-4 strain 341750 PDK-6 having the ATCC accession number PTA-4811.

produced by serial passaging of an infectious dengue virus isolate in a suitable host cell line such as primary dog kidney cells so that mutations accumulate that confer attenuation on the isolate. Serial passaging refers to the infection of a cell line with a virus isolate, the recovery of the viral progeny from the host cells, and the subsequent infection of host cells with the viral progeny to generate the next passage.

Preferably, the following attenuated viruses are used in the compositions of the present invention even though other virus compositions, of any of the serotypes, whether attenuated or inactivated, can be used in combination with the attenuated strains described in the present invention. The attenuated dengue-1 virus, derived from 45AZ5 isolate, deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) of 10801 University Boulevard, Manassas, Virginia 20110-2209, U.S.A., and granted the accession number of VR-2648.

The attenuated dengue-2 virus derived from S16803 isolate, deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) of 10801 University Boulevard, Manassas, Virginia 20110-2209, U.S.A., and granted the accession number of VR-2653.

The attenuated dengue-3 virus derived from CH53489 isolate, deposited under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) of 10801 University Boulevard, Manassas, Virginia 20110-2209, U.S.A., and granted the accession number of VR-2647.

The attenuated dengue-4 virus derived from the 341750 isolate, deposited under the terms of the Budapest Treaty with the American Type Culture

Collection (ATCC) of 10801 University Boulevard, Manassas, Virginia 20110-2209, U.S.A., and granted the accession number of VR-2652.

Serial passaging of a virulent (disease-causing) strain of dengue results in the isolation of modified virus which may be attenuated, i.e., infectious, yet not capable of causing disease. These modified viruses are tested in monkeys for reduced infectivity. Those that have reduced infectivity are subsequently tested in humans. Humans are the only primate that will exhibit signs of clinical disease. The viruses that show minimal to no clinical reactivity but still infect and induce an immune response are attenuated.

In one embodiment of the invention, a virulent dengue isolate from all four dengue serotypes was serially passaged in primary dog kidney (PDK) cells to derive the attenuated strains. Serial passaging was performed by infecting PDK cells with the virulent strain, incubating the infected cells for several days, and collecting the supernatant culture fluids containing virus. The harvested virus was then applied to fresh PDK cells to generate the next passage.

Various passages in the series were tested for clinical effect after final passage in fetal Rhesus monkey lung cells (FRhL). FRhL cells were used to optimize virus titers wherein, in general, passage 1 was considered the master seed, passage 2 was considered the production seed, and passage 3 was considered the vaccine lot. Vaccines were prepared at various PDK passage levels, and the vaccine products tested for attenuation in monkeys and humans. The virulence of a passaged virus, i.e., the ability to cause disease, was assessed by daily monitoring of symptoms such as temperature (fever), headache, rash,

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USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2006


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E1            4     ECKELS JOSHUA MOLL/IN
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E3            2 --> ECKELS KENNETH/IN
E4            9     ECKELS KENNETH H/IN
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E6            3     ECKELS PAUL/IN
E7           23     ECKELS PHILLIP W/IN
E8           12     ECKELS PHILLIP WILLIAM/IN
E9            2     ECKELS RICHARD EDGAR/IN
E10          22     ECKELS ROBERT E/IN
E11           1     ECKELS ROBERT EDWARD/IN
E12           2     ECKELS ROBERT Y/IN

=> s e3-e4
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              9 "ECKELS KENNETH H"/IN
L1           11 ("ECKELS KENNETH"/IN OR "ECKELS KENNETH H"/IN)

=> d l1,cbib,clm,1-11

L1   ANSWER 1 OF 11  USPATFULL on STN
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US 6676936 B1 20040113
APPLICATION: US 2000-643217 20000818 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A recombinant genetic construct, adapted to encode a chimeric flaviviral genome, comprising: a full genome-length nucleic acid clone of a flavivirus genome having a region of nucleic acid encoding structural protein of a first flavivirus linked to a region of nucleic acid encoding non-structural protein of a second flavivirus, wherein said second flavivirus is a different flavivirus from said first flavivirus.

2. The genetic construct of claim 1 comprising DNA.

3. A method of inducing an immune response in a host against a first flavivirus comprising: (a) preparing the genetic construct of claim 1, wherein said genetic construct comprises DNA; (b) generating infectious RNA transcripts from said DNA construct; (c) introducing said RNA transcripts into a cell; (d) expressing said RNA transcripts in said cell to produce virus; (e) harvesting said virus from said cell; (f) testing said virus in an animal model; and (g) inoculating said host with virus produced by repeating steps (a)-(e).

4. A chimeric virus having a genome comprising: a full genome-length nucleic acid clone of a flavivirus genome having a region of nucleic acid encoding structural protein of a first flavivirus linked to a region of nucleic acid encoding non-structural protein of a second flavivirus, wherein said second flavivirus is a different flavivirus from said first flavivirus.

5. An immunogenic composition against a first flavivirus comprising the chimeric virus of claim 4 and a pharmaceutically acceptable carrier.

L1 ANSWER 2 OF 11 USPATFULL on STN
2003:285090 Multivalent dengue virus vaccine.
Eckels, Kenneth H., Rockville, MD, United States
Putnak, Joseph R., Silver Spring, MD, United States
Dubois, Doria R., Wheaton, MD, United States
Innis, Bruce L., Haverford, PA, United States
Hoke, Charles H., Columbia, MD, United States
Wellington, Sun, Rockville, MD, United States
Kanessa-thasan, Niranjan, Rockville, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6638514 B1 20031028
APPLICATION: US 2000-535117 20000324 (9)
PRIORITY: US 2000-181724P 20000211 (60)
US 1999-126313P 19990326 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. An immunogenic composition comprising two or more attenuated dengue viruses selected from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652, and a physiologically acceptable vehicle.

2. The immunogenic composition according to claim 1 which further comprises an adjuvant to enhance the immune response.

3. The immunogenic composition of claim 1, formulated in a dose of 10^2 to 10^6 PFU of attenuated virus.

4. A multivalent live attenuated dengue virus vaccine comprising any combination of dengue virus serotypes selected from the group consisting of: a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a dengue-2 (DEN-2)

accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652.

5. The dengue virus vaccine of claim 4 wherein said dengue virus is produced in vertebrate cells.

6. The dengue virus vaccine of claim 5 wherein said cells are Vero cells.

7. The dengue virus vaccine of claim 4 wherein said dengue-1 virus is in the amount of 10^2 to 10^7 pfu/ml, said dengue-2 virus is in the amount of 10^2 to 10^7 pfu, said dengue-3 virus is in the amount of 10^2 to 10^7 pfu, and said dengue-4 virus is in the amount of 10^2 to 10^7 pfu/ml.

8. The dengue virus vaccine of claim 7 wherein said vaccine is administered subcutaneously.

9. An immunogenic composition comprising two or more attenuated dengue virus chosen from the group consisting of a dengue-1 (DEN-1) virus having the sequence of DEN-1 sawn 45AZ5 PDK-27 having the ATCC accession number PTA4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-S0 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH153489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN4) virus having the sequence of DEN-4 strain 341750 PDK6 having the ATCC accession number PTA4811, and a physiologically acceptable vehicle.

10. A multivalent live attenuated dengue virus vaccine comprising any combination of dengue virus serotypes selected from the group consisting of: a dengue-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA4810, a dengue-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a dengue-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a dengue-4 (DEN-4) virus having the sequence of DEN-4 stain 341750 PDK-6 having the ATCC accession number PTA-4811.

11. The vaccine of claim 10 wherein at least one virus os DEN-1 strain 45Az5 PDK-27 having the ATCC accessior number PTA-4810.

12. The vaccine of claim 10 wherein at least one virus is DEN-4 strain 341750 PDK-6 having the ATCC accessior number PTA-4811.

L1 ANSWER 3 OF 11 USPATFULL on STN
2003:234689 Adaptation of virus to vertebrate cells.
Eckels, Kenneth H., Rockville, MD, United States
Putnak, Joseph R., Silver Spring, MD, United States
Innis, Bruce L., Haverford, PA, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6613556 B1 20030902
APPLICATION: US 2000-534724 20000324 (9)
PRIORITY: US 1999-126316P 19990326 (60)
US 2000-182065P 20000211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CLM What is claimed is:
1. A method for replicating dengue virus to a titer growth of at least 10^5 PFU/ml, wherein the dengue virus is selected from the group consisting of the dengue-1 strain identified as 45AZ5 PDK20 and given the ATCC accession no. VR-2648; the dengue-2 strain identified as S16803 PDK50 and given the ATCC accession no. VR-2653, the dengue 3 strain identified as CH53489 PDK20 and given the ATCC accession no. VR-2647, and the dengue 4 strain identified as 341750 PDK20 and given the ATCC accession no. VR-2652, comprising the step of infecting cells from a continuous epithelial or fibroblast cell line with a growth strain having a titer growth of at least 10^5 PFU/ml, wherein said continuous epithelial or fibroblast cell line lacks contaminating adventitious agents such that cells from said cell line are suitable for use in mammalian virus vaccine production.

2. The method of claim 1 wherein the dengue virus is chosen from the group consisting of: dengue 1, dengue 2, dengue 3, and dengue 4.

3. The method according to claim 2 wherein said dengue virus is attenuated.

4. The method of claim 1 wherein said cells are Vero cells.

of 20-190.

L1 ANSWER 4 OF 11 USPATFULL on STN
2003:105804 Microfluidized leishmania lysate and methods of making and using thereof.
Magill, Alan J., Kensington, MD, UNITED STATES
Stiteler, John M., Springfield, VA, UNITED STATES
Grogl, Max, Columbia, MD, UNITED STATES
Rowton, Edgar D., College Park, MD, UNITED STATES
Eckels, Kenneth H., College Park, MD, UNITED STATES
Ballou, William R., Silver Spring, MD, UNITED STATES
US 2003072714 A1 20030417
APPLICATION: US 2001-975020 A1 20011012 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A method of preparing a microfluidized lysate preparation comprising microfluidizing a slurry of at least one Leishmania parasite through a chamber and disrupting the leishmania parasite with a sudden release of pressure.

2. The method of claim 1, further comprising heat treating the microfluidized lysate preparation.

3. The method of claim 1, wherein the Leishmania parasite is L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum, or L. aethiopica.

4. A microfluidized lysate preparation made by the method of claim 1.

5. A skin test antigen assay for detecting whether a subject had been exposed to a Leishmania parasite or was afflicted with Leishmaniasis comprising administering to the subject an antigenic amount of at least one microfluidized lysate preparation according to claim 4 and observing any immunogenic response to the microfluidized lysate preparation.

6. The skin test antigen assay of claim 5, wherein the Leishmania parasite is L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum, or L. aethiopica.

7. The skin test antigen assay of claim 5, wherein an immunogenic response indicates that the subject had been exposed to a Leishmania parasite or was afflicted with Leishmaniasis.

8. The skin test antigen assay of claim 5, wherein an induration of about 5 mm or greater observed indicates that the subject had been exposed to a Leishmania parasite or was afflicted with Leishmaniasis.

9. The skin test antigen assay of claim 5, wherein the antigenic amount of the microfluidized lysate preparation comprises about 5 μg to about 30 μg of total protein.

10. The skin test antigen assay of claim 5, wherein the antigenic amount of the microfluidized lysate preparation is administered intradermally to the volar surface of the forearm of the subject.

11. A kit comprising the microfluidized lysate preparation of claim 4 and directions for determining whether a subject has been exposed to a Leishmania parasite or was afflicted with Leishmaniasis.

12. The kit of claim 11, wherein the Leishmania parasite is L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum, or L. aethiopica.

13. The kit of claim 11, further comprising at least one pharmaceutical for treating systemic anaphylaxis.

14. The kit of claim 13, wherein the pharmaceutical is epinephrine, diphenhydramine, or methyl prednisolone.

15. The kit of claim 11, further comprising at least one pharmaceutical for treating local reactions to the microfluidized lysate preparation.

16. The kit of claim 15, wherein the pharmaceutical is hydrocortisone, hydrocortisone cream, acetaminophen, or diphenhydramine.

17. An antibody raised against the microfluidized lysate preparation of claim 4.

18. A vaccine comprising the microfluidized lysate preparation of claim 4.

19. A method of determining whether a subject has been exposed to a given Leishmania parasite comprising administering to the subject a panel of antigenic compositions comprising a plurality of microfluidized lysate preparations prepared from a plurality of Leishmania parasites and detecting a presence of an immunogenic reaction that is characteristic to exposure to the given Leishmania parasite.

20. The method of claim 19, wherein the plurality of Leishmania parasites comprises at least one parasite from the group consisting of L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum, and L. aethiopica.

21. A method of immunizing a subject against Leishmaniasis comprising administering to the subject an immunogenic amount of the microfluidized lysate preparation of claim 4.

22. A pharmaceutical composition comprising the microfluidized lysate preparation of claim 4 and a pharmaceutically acceptable stabilizer.

23. The pharmaceutical composition of claim 22, wherein the pharmaceutically acceptable stabilizer is phenol.

24. The pharmaceutical composition of claim 22, wherein the composition is in the form of a liquid.

25. The pharmaceutical composition of claim 22, wherein the composition may be frozen or freeze-dried.

26. A method for determining post infection of cutaneous leishmaniasis, mucocutaneous leishmaniasis, or post-kala-azar dermal leishmaniasis in a subject comprising administering to the subject an antigenic amount of at least one microfluidized lysate preparation of claim 4 and observing any immunogenic response to the microfluidized lysate preparation.

27. A method for epidemiologically diagnosing cutaneous leishmaniasis, mucocutaneous leishmaniasis, or post-kala-azar dermal leishmaniasis in a subject comprising administering to the subject an antigenic amount of at least one microfluidized lysate preparation of claim 4 and observing any immunogenic response to the microfluidized lysate preparation.

28. A method for determining the pattern of present and past leishmaniasis in a subject comprising administering to the subject an antigenic amount of at least one microfluidized lysate preparation of claim 4 and observing any immunogenic response to the microfluidized lysate preparation.

L1 ANSWER 5 OF 11 USPATFULL on STN
2003:81459 Attenuated dengue-4 virus vaccine.
Eckels, Kenneth H., Rockville, MD, United States
Putnak, Joseph R., Silver Spring, MD, United States
Dubois, Doria R., Wheaton, MD, United States
Innis, Bruce L., Haverford, PA, United States
Hoke, Charles H., Columbia, MD, United States
Vaughn, David, Silver Spring, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6537557 B1 20030325
APPLICATION: US 2000-534726 20000324 (9)
PRIORITY: US 1999-126318P 19990326 (60)
US 2000-182068P 20000211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An immunogenic composition comprising an attenuated dengue-4 virus strain 341750 PDK-6, in a physiologically acceptable vehicle.

2. The immunogenic composition according to claim 1, which induces a dengue-4 specific immune response in individuals.

3. The immunogenic composition of claim 1, formulated in a dose of 10^4 to 10^5 PFU of attenuated virus.

L1 ANSWER 6 OF 11 USPATFULL on STN
2003:59949 Attenuated dengue-3 virus vaccine.
Eckels, Kenneth H., Rockville, MD, United States
Putnak, Joseph R., Silver Spring, MD, United States

Innis, Bruce L., Hayerford, PA, United States
Hoke, Charles H., Columbia, MD, United States
Vaughn, David, Silver Spring, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6528065 B1 20030304
APPLICATION: US 2000-535684 20000324 (9)
PRIORITY: US 2000-182063P 20000211 (60)
US 1999-126311P 19990326 (60)
DOCUMENT TYPE: Utility; GRANTED.

CLM What is claimed is:

1. An immunogenic composition comprising, in a physiologically acceptable vehicle, at least one attenuated dengue-3 virus having the sequence of the virus designated ATCC accession number VR-2647.

2. The immunogenic composition according to claim 1, which induces a dengue-3 specific immune response in individuals.

3. The immunogenic composition of claim 1, formulated in a dose of 10^4 to 10^5 PFU of attenuated virus.

L1 ANSWER 7 OF 11 USPATFULL on STN

2003:26150 Attenuated dengue-2 virus vaccine.

Eckels, Kenneth H., Rockville, MD, United States
Putnak, Joseph R., Silver Spring, MD, United States
Dubois, Doria R., Wheaton, MD, United States
Innis, Bruce L., Haverford, PA, United States
Hoke, Charles H., Columbia, MD, United States
Vaughn, David, Silver Spring, MD, United States
Henchai, Erik A., Rockville, MD, United States
Kanesa-thasan, Niranian, Rockville, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6511667 B1 20030128
APPLICATION: US 2000-534725 20000324 (9)
PRIORITY: US 1999-126319P 19990326 (60)
US 2000-182067P 20000211 (60)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. An immunogenic composition comprising, in a physiologically acceptable vehicle, at least one attenuated dengue-2 virus having the sequence of the virus designated ATCC accession number VR-2653.

2. The immunogenic composition according to claim 1, which induces a dengue-2 specific immune response in individuals.

3. The immunogenic composition of claim 1, formulated in a dose of 10^4 to 10^5 PFU of attenuated virus.

L1 ANSWER 8 OF 11 USPATFULL on STN

2001:190736 Attenuated Japanese encephalitis virus adapted to Vero cell and a Japanese encephalitis vaccine.

Kim, Hyun Su, Seoul, Korea, Republic of
Yoo, Wang Don, Seoul, Korea, Republic of
Kim, Soo Ok, Seoul, Korea, Republic of
Lee, Sung Hee, Kyungkwido, Korea, Republic of
Moon, Sang Bum, Kyungkwido, Korea, Republic of
Hong, Sun Pyo, Kyungkwido, Korea, Republic of
Shin, Yong Cheol, Seoul, Korea, Republic of
Chung, Yong Ju, Seoul, Korea, Republic of
Eckels, Kenneth H., Washington, DC, United States
Innis, Bruce, Washington, DC, United States
Puniak, Joseph R., Washington, DC, United States
Binn, Leonard N., Washington, DC, United States
Srivastava, Ashok K., Washington, DC, United States
Dubois, Doria R., Washington, DC, United States
Cheil Jedang Corporation, Seoul, Korea, Republic of (non-U.S. corporation)The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6309650 B1 20011030
WO 9911762 19990311
APPLICATION: US 2000-486392 20000615 (9)
WO 1998-KR259 19980825 20000615 PCT 371 date 20000615 PCT 102(e) date
PRIORITY: KR 1997-42001 19970828
KR 1997-42002 19970828
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

CLM What is claimed is:

1. An attenuated Japanese encephalitis virus adapted to Vero cell by

1×10^{7} PFU/ml in Vero cells and LD_{50} /pfu for young adult mouse is less than 0.000001.

2. A Japanese encephalitis vaccine comprising the attenuated Japanese encephalitis virus according to claim 1.

3. The vaccine according to claim 2, which further comprises pharmaceutically acceptable additives.

4. The vaccine according to claim 2 wherein the virus is inactivated by an inactivating. agent.

5. The vaccine according to claim 4, which further comprises pharmaceutically acceptable additives.

6. The vaccine according to claim 2 wherein the virus is live-attenuated JE virus untreated by an inactivating agent.

7. The vaccine according to claim 6, which further comprises pharmaceutically acceptable additives.

8. An attenuated Japanese encephalitis virus adapted to Vero cell by passages on Vero cell which is CJ50003.

9. A Japanese encephalitis vaccine comprising the attenuated Japanese encephalitis virus according to claim 8.

10. The vaccine according to claim 8, wherein the virus is inactivated by an inactivating agent.

11. The vaccine according to claim 8, wherein the virus is live-attenuated JE virus untreated by an inactivating agent.

12. The vaccine according to claim 9, which further comprises pharmaceutically acceptable additives.

L1 ANSWER 9 OF 11 USPATFULL on STN
2001:102384 Inactivated dengue virus vaccine.
Putnak, J. Robert, Silver Spring, MD, United States
Eckels, Kenneth, Rockville, MD, United States
Dubois, Doris R., Wheaton, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6254873 B1 20010703
APPLICATION: US 1995-423338 19950417 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A vaccine comprising a purified, inactivated dengue virus effective for inducing a protective immune response in primates against dengue virus.

2. The purified, inactivated dengue virus vaccine of claim 1 comprising a first inactivated dengue virus, wherein said inactivated dengue virus is selected from the group consisting of dengue virus type 1, dengue virus type 2, dengue virus type 3, and dengue virus type 4.

3. The purified, inactivated dengue virus vaccine of claim 2, wherein said dengue virus vaccine further comprises of one or more purified inactivated dengue virus serotype(s) different from the first inactivated dengue virus, wherein said purified, inactivated dengue virus is selected from the group consisting of dengue virus type 1, dengue virus type 2, dengue virus type 3 and dengue virus type 4.

4. A method for producing antibodies against dengue virus comprising administering to an individual a purified, inactivated dengue virus vaccine according to claim 1 in an amount sufficient to produce said antibodies wherein said vaccine is chosen from the group consisting of dengue virus type 1 vaccine, dengue virus type 2 vaccine, dengue virus type 3 vaccine and dengue virus type 4 vaccine.

5. A method for producing the purified, inactivated dengue virus vaccine of claim 1 comprising the steps of: (i) propagating dengue virus in a cell culture; (ii) harvesting said virus from said cell culture; (iii) concentrating said virus; (iv) purifying said virus such that it is essentially free of cell culture proteins and DNA; (v) inactivating said virus; and (vi) adding a suitable adjuvant in a pharmaceutically acceptable amount.

6. A method for producing a purified, inactivated dengue virus vaccine according to claim 5, further comprising the step of removing serum

(ii).

7. The purified inactivated dengue virus vaccine of claim 1 wherein said inactivated dengue virus is chemically inactivated.

8. The purified, inactivated dengue virus vaccine of claim 7 comprising a first inactivated dengue virus, wherein said first inactivated dengue virus is selected from the group consisting of dengue virus type 1, dengue virus type 2, dengue virus type 3, and dengue virus type 4.

9. The purified, inactivated dengue virus vaccine of claim 8, wherein said dengue virus vaccine further comprises an additional one or more purified inactivated dengue virus serotype(s) different from said first inactivated dengue virus, wherein said additional inactivated dengue virus is selected from the group consisting of dengue virus type 1, dengue virus type 2, dengue virus type 3, and dengue virus type 4.

10. The purified, inactivated dengue virus vaccine according to claim 1 wherein the specific activity of said vaccine is at least about 1×10^8 plaque forming units per milligram of total protein.

11. The purified inactivated dengue virus vaccine of claim 1, wherein said dengue virus vaccine is dengue 1 virus vaccine produced from deposited dengue 1 virus having ATCC accession no. VR-2649.

12. The purified inactivated dengue virus vaccine of claim 1, wherein said dengue virus vaccine is dengue 2 virus vaccine produced from deposited dengue 2 virus having ATCC accession no. VR-2650.

13. The purified inactivated dengue virus vaccine of claim 1, wherein said dengue virus vaccine is dengue 3 virus vaccine produced from deposited dengue 3 virus having ATCC accession no. VR-2654.

14. The purified inactivated dengue virus vaccine of claim 1, wherein said dengue virus vaccine is dengue 4 virus vaccine produced from deposited dengue 4 virus having ATCC accession no. VR-2651.

15. A method for inducing an immune response in an individual against dengue virus comprising administering to an individual a purified, inactivated dengue virus vaccine according to claim 1 in an amount sufficient to produce said immune response wherein said vaccine is chosen from the group consisting essentially of dengue virus type 1 vaccine, dengue virus type 2 vaccine, dengue virus type 3 vaccine and dengue virus type 4 vaccine or any combination thereof.

16. A dengue 2 virus vaccine produced by inactivating a dengue 2 virus deposited at ATCC under accession no. VR2650.

17. A multivalent vaccine comprising purified inactivated dengue virus propagated in vertebrate tissue culture cells and effective for inducing a protective immune response in primates against dengue virus wherein said virus is chosen from the group consisting of purified inactivated dengue virus type 1, purified inactivated dengue virus type 2, purified inactivated dengue virus type 3, and purified inactivated dengue virus type 4.

18. An isolated dengue type 1 virus having ATCC accession number VR-2649.

19. An isolated dengue type 1 virus vaccine produced by inactivating a dengue 1 virus of claim 18.

20. An isolated cell infected with the dengue type 1 virus of claim 18.

21. An isolated dengue type 2 virus having ATCC accession number VR-2650.

22. An isolated cell infected with the dengue type 2 virus of claim 21.

23. An isolated dengue type 3 virus having ATCC accession number VR-2654.

24. An isolated dengue type 3 virus vaccine produced by inactivating a dengue 3 virus of claim 23.

25. An isolated cell infected with the dengue type 3 virus of claim 23.

26. An isolated dengue type 4 virus having ATCC accession number VR-2651.

27. An isolated dengue type 4 virus vaccine produced by inactivating a dengue 4 virus of claim 26.

28. An isolated cell infected with the dengue type 4 virus of claim 26.

L1 ANSWER 10 OF 11 USPATFULL on STN
2001:25624 Method and kit for detection of dengue virus.
Putnak, J. Robert, Silver Spring, MD, United States
Eckels, Kenneth, Rockville, MD, United States
Dubois, Doria R., Wheaton, MD, United States
Cassidy, Kevin, Toronto, Canada
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6190859 B1 20010220
APPLICATION: US 1995-422458 19950417 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for detecting an antibody directed against a dengue virus antigen comprising: (i) incubating a sample suspected of containing anti-dengue antibodies with purified inactivated dengue virus wherein said inactivated dengue virus is one or more purified inactivated dengue virus selected from the group consisting of dengue virus type 1 having accession no. VR-2649, dengue virus type 2 having accession no. VR-2650, dengue virus type 3 having accession no. VR-2654, and dengue virus type 4 having accession no. VR-2651 under conditions which allow the formation of an antibody-inactivated virus complex; and (ii) detecting said antibody by detecting the antibody-inactivated virus complex.

2. A diagnostic kit for the detection of antibodies against dengue virus antigens in a sample, said kit comprising one or more purified, inactivated dengue virus selected from the group consisting of dengue virus type 1 having accession no. VR-2649, dengue virus type 2 having accession no. VR-2650, dengue virus type 3 having accession no. VR-2654, and dengue virus type 4 having accession no. VR-2651, for use in detecting said antibodies.

3. A method for detecting an antibody directed against a dengue virus antigen according to claim 1, wherein said inactivated virus is chemically inactivated.

4. The diagnostic kit according to claim 2 wherein said inactivated dengue virus is chemically inactivated.

5. The method according to claim 1 wherein said dengue virus antigen is a dengue virus type 1 antigen, and said inactivated dengue virus is dengue virus type 1 having ATCC accession no. VR-2649.

6. The method according to claim 1 wherein said dengue virus antigen is a dengue virus type 2 antigen, and said inactivated dengue virus is dengue virus type 2 having ATCC accession no. VR-2650.

7. The method according to claim 1, wherein said dengue virus antigen is a dengue virus type 3 antigen, and said inactivated dengue virus is dengue virus type 3 having ATCC accession no. VR-2654.

8. The method according to claim 1, wherein said dengue virus antigen is a dengue virus type 4 antigen, and said inactivated dengue virus is dengue virus type 4 having ATCC accession no. VR-2651.

L1 ANSWER 11 OF 11 USPATFULL on STN
2001:18271 Chimeric and/or growth-restricted flaviviruses.
Lai, Ching-Juh, Bethesda, MD, United States
Bray, Michael, Bethesda, MD, United States
Pletnev, Alexander G., Rockville, MD, United States
Men, Ruhe, Kensington, MD, United States
Zhang, Yi-Ming, Bethesda, MD, United States
Eckels, Kenneth H., Bethesda, MD, United States
Chanock, Robert M., Bethesda, MD, United States
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6184024 B1 20010206
APPLICATION: US 1994-250802 19940527 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A recombinant genetic construct, adapted to encode a chimeric flaviviral genome, comprising: a full genome-length nucleic acid clone of a flavivirus genome having a region of nucleic acid encoding structural protein of a first flavivirus linked to a region of nucleic acid encoding non-structural protein of a second flavivirus, wherein said second flavivirus is a different flavivirus from said first flavivirus, and wherein said flavivirus is defined as an approximately

open reading frame for three structural proteins, capsid (C), premembrane (preM) and envelope (E), followed by seven non-structural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

2. The genetic construct of claim 1, wherein said second flavivirus is a dengue virus.

3. The genetic construct of claim 2, wherein said first flavivirus is a member selected from the group consisting of dengue type 1 virus, dengue type 2 virus, and dengue type 3 virus, and said second flavivirus is dengue type 4 virus.

4. The genetic construct of claim 2 further comprising at least one mutation that is introduced into the viral genome.

5. The genetic construct of claim 4, wherein said mutation is a member selected from the group consisting of one or more mutations that reduce glycosylation of premembrane protein, envelope protein or NS1(1) protein; one or more mutations that reduce cleavage of premembrane protein to membrane protein; one or more substitutions at a site encoding glycine, which site is at position +1 following polyprotein NS1-NS2A cleavage site; one or more deletions comprising at least 30 nucleotides between nucleotide 113 and 384 inclusive, number 1 being a 3'-most nucleotide of a 3'-non-coding end; and one or more mutations in a sequence encoding one or more of eight amino acids at the carboxy terminus cleavage site of NS1.

6. The genetic construct of claim 1, wherein said first flavivirus is a member selected from the group consisting of type 1 dengue virus, type 2 dengue virus, type 3 dengue virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, and said second flavivirus is type 4 dengue virus.

7. The genetic construct of claim 1, wherein said first flavivirus is a member selected from the group consisting of type 1 dengue virus, type 2 dengue virus, type 3 dengue virus, type 4 dengue virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, and said second flavivirus is a member selected from the group consisting of type 1 dengue virus, type, 2 dengue virus, and type 3 dengue virus.

8. The genetic construct of claim 1, wherein said first flavivirus is a member selected from the group consisting of type 1 dengue virus, type 2 dengue virus, type 3 dengue virus, type 4 dengue virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, and said second flavivirus is a member selected from the group consisting of yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus.

9. The genetic construct of claim 1, wherein said region of nucleic acid encoding structural protein recodes premembrane protein and enevlope protein of tick-borne encephalitis virus, and further comprising a region of nucleic acid encoding capsid protein from said second flavivirus.

10. The genetic construct of claim 9, wherein said second flavivirus is a dengue virus.

11. The genetic construct of claim 1, wherein said region of nucleic acid encoding structural protein encodes premembrane protein and envelope protein of Japanese encephalitis virus, and further comprising a region of nucleic acid encoding capsid protein from said second flavivirus.

12. The genetic construct of claim 11, wherein said second flavivirus is a dengue virus.

13. A method of inducing an immune response in a host against a first flavivirus comprising: (a) preparing the genetic construct of any of claims 1-12, wherein said genetic construct comprises DNA; (b) generating infectious RNA transcripts from said DNA construct; (c) introducing said RNA transcripts into a cell; (d) expressing said RNA transcripts in said cell to produce virus; (e) harvesting said virus from said cell; (f) testing said virus in an animal model; and (g) inoculating said host with virus produced by repeating steps (a)-(e).

14. The genetic construct of claim 1, comprising DNA.

15. A chimeric virus having a genome comprising: a full genome-length nucleic acid clone of a flavivirus genome having a region of nucleic acid encoding structural protein of a first flavivirus linked to a region of nucleic acid encoding non-structural protein of a second

from said first flavivirus, and wherein said flavivirus is defined as an approximately 11 -kilobase positive strand RNA virus having a genome that codes in one open reading frame for three structural proteins, capsid (C), premembrane (preM) and envelope (E), followed by seven non-structural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

16. The chimeric virus of claim 15, wherein said second flavivirus is a dengue virus.

17. The chimeric virus of claim 16, wherein said first flavivirus is a member selected from the group consisting of dengue type 1 virus, dengue type 2 virus, and dengue type 3 virus, and said second flavivirus is dengue type 4 virus.

18. The chimeric virus of claim 15 further comprising at least one mutation that is introduced into the viral genome.

19. The chimeric virus of claim 18, wherein said mutation is a member selected from the group consisting of one or more mutations that reduce glycosylation of premembrane protein, envelope protein or NS1(1) protein; one or more mutations that reduce cleavage of premembrane protein to membrane protein; one or more substitutions at a site encoding glycine, which site is at position +1 following polyprotein NS1-NS2A cleavage site; one or more deletions comprising at least 30 nucleotides between nucleotide 113 and 384 inclusive, number 1 being a 3'-most nucleotide of a 3'-non-coding end; and one or more mutations in a sequence encoding one or more of eight amino acids at the carboxy terminus cleavage site of NS1.

20. The chimeric virus of claim 15, wherein said first flavivirus is a member selected from the group consisting of type 1 dengue virus, type 2 dengue virus, type 3 dengue virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, and said second flavivirus is type 4 dengue virus.

21. The chimeric virus of claim 15, wherein said first flavivirus is a member selected from the group consisting of type 1 dengue virus, type 2 dengue virus, type 3 dengue virus, type 4 dengue virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, and said second flavivirus is a member selected from the group consisting of type 1 dengue virus, type 2 dengue virus, and type 3 dengue virus.

22. The chimeric virus of claim 15, wherein said first flavivirus is a member selected from the group consisting of type 1 dengue virus, type 2 dengue virus, type 3 dengue virus, type 4 dengue virus, yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus, and said second flavivirus is a member selected from the group consisting of yellow fever virus, Japanese encephalitis virus, and tick-borne encephalitis virus.

23. The chimeric virus of claim 15, wherein said region of nucleic acid encoding structural protein encodes premembrane protein and envelope protein of tick-bome encephalitis virus, and further comprising a region of nucleic acid encoding capsid protein from said second flavivirus.

24. The chimeric virus of claim 23, wherein said second flavivirus is a dengue virus.

25. The chimeric virus of claim 15, wherein said region of nucleic acid encoding structural protein encodes premembrane protein and envelope protein of Japanese encephalitis virus, and further comprising a region of nucleic acid encoding capsid protein from said second flavivirus.

26. The chimeric virus of claim 25, wherein said second flavivirus is a dengue virus.

27. An immunogenic composition against a first flavivrus comprising the chimeric virus of any of claims 15-26 and a pharmaceutically acceptable carrier.

28. An isolated recombinant DNA construct that encodes stable, full-length infectious dengue type 4 viral RNA.

29. The DNA construct of claim 28, further comprising a vector.

30. The DNA construct of claim 29, wherein said vector is a plasmid.

31. A host cell stably transformed with the DNA construct of claim 29, in a manner allowing expression of said DNA construct.

32. The host cell of claim 31, wherein said host cell is a prokaryotic

33. The DNA construct of claim 28, further comprising at least one mutation that is introduced into the viral genome.

34. The DNA construct of claim 33, wherein said mutation is a member selected from the group consisting of one or more mutations that reduce glycosylation of premembrane protein, envelope protein or NS1(1) protein; one or more mutations that reduce cleavage of premembrane protein to membrane protein; one or more substitutions at a site encoding glycine, which site is at position +1 following polyprotein NS1-NS2A cleavage site; one or more deletions comprising at least 30 nucleotides between nucleotide 113 and 384 inclusive, number 1 being a 3'-most nucleotide of a 3'-non-coding end; and one or more mutations in a sequence encoding one or more of eight amino acids at the carboxy terminus cleavage site of NS1.

35. An immunogenic composition against dengue type 4 virus comprising a dengue type 4 virus, having a stable, full-length infectious dengue type 4 viral genome comprising at least one mutation that is introduced into the viral genome, and a pharmaceutically acceptable carrier.

36. The immunogenic composition of claim 35, wherein said mutation is a member selected from the group consisting of one or more mutations that reduce glycosylation of premembrane protein, envelope protein or NS1(1) protein; one or more mutations that reduce cleavage of premembrane protein to membrane protein; one or more substitutions at a site encoding glycine, which site is at position +1 following polyprotein NS1-NS2A cleavage site; one or more deletions comprising at least 30 nucleotides between nucleotide 113 and 384 inclusive, number 1 being a 3'-most nucleotide of a 3'-non-coding end; and one or more mutations in a sequence encoding one or more of eight amino acids at the carboxy terminus cleavage site of NS1.

37. An isolated RNA segment comprising stable, full-length infectious dengue type 4 viral genome transcribed from a recombinant DNA construct.

38. The RNA segment of claim 37, further comprising at least one mutation that is introduced into the viral genome.

39. The RNA segment of claim 38, wherein said mutation is a member selected from the group consisting of one or more mutations that reduce glycosylation of premembrane protein, envelope protein or NS1(1) protein; one or more mutations that reduce cleavage of premembrane protein to membrane protein; one or more substitutions at a site encoding glycine, which site is at position +1 following polyprotein NS1-NS2A cleavage site; one or more deletions comprising at least 30 nucleotides between nucleotide 113 and 384 inclusive, number 1 being a 3'-most nucleotide of a 3'-non-coding end; and one or more mutations in a sequence encoding one or more of eight amino acids at the carboxy terminus cleavage site of NS1.

40. A baculovirus, comprising: a 4.0 kilobase recombinant dengue cDNA sequence that encodes dengue virus capsid protein, pre-matrix protein, envelope glycoprotein, and NS1 and NS2a nonstructural proteins.

41. An immunogenic composition consisting essentially of dengue type 4 virus envelope glycoprotein and NS1 nonstructural protein.

42. An immunogenic composition consisting essentially of dengue type 4 virus capsid protein, pre-matrix protein, envelope glycoprotein and NS1 nonstructural protein.

43. A baculovirus comprising the dengue cDNA coding sequence for: (1) only dengue envelope glycoprotein or (2) only dengue non-structural proteins NS1 and NS2a.

44. An immunogenic composition consisting essentially of: a pharmaceutically acceptable carrier; and an amount of dengue type 4 virus proteins effective to induce a dengue type 4 virus specific immunological response.

45. The composition of claim 44, wherein said dengue type 4 virus proteins are a member selected from the group consisting of dengue type 4 virus capsid protein, pre-matrix protein, envelope glycoprotein, NS1 nonstructural protein, NS2a nonstructural protein, and mixtures of these.

46. A method for producing an immunogenic composition, comprising: culturing cells infected with a baculovirus comprising a nucleotide sequence encoding at least one dengue type 4 virus protein under conditions such that said dengue type 4 virus protein is produced; separating said dengue type 4 virus protein from said cells; and

therapeutically effective concentration with a pharmaceutically effective carrier.

47. The method of claim 46, wherein said dengue type 4 virus protein is a member selected from the group consisting of dengue type 4 virus capsid protein, pre-matrix protein, envelope glycoprotein, NS1 nonstructural protein, NS2a nonstructural protein, and mixtures of these.

48. The method of claim 46, wherein said infected cells are Spodoptera frugiperda cells.

49. A baculovirus comprising the Japanese B encephalitis virus cDNA coding sequence for the 3 structural proteins (C-M-E) and non-structural proteins NS1 and NS2a.

50. A method for producing an immunogenic composition, comprising: culturing a recombinant baculovirus in infected cells to express Japanese B encephalitis protein; separating said Japanese B encephalitis protein; and formulating said Japanese B encephalitis protein in a therapeutically effective concentration with a pharmaceutically effective carrier.

51. The method of claim 50, wherein said Japanese B encephalitis protein is a member selected from the group consisting of Japanese B encephalitis capsid protein, pre-matrix protein, envelop glycoprotein, NS1 nonstructural protein, NS2a nonstructural protein, and mixtures of these.

52. The method of claim 50, where said infected cells are Spodoptera frugiperda cells.

```
=> e putnak joseph r/in
E1            7     PUTNA ERNISSE S/IN
E2            3     PUTNAK J ROBERT/IN
E3            6 --> PUTNAK JOSEPH R/IN
E4            1     PUTNAL CLIFFORD E/IN
E5            2     PUTNAM ABBOTT A/IN
E6            4     PUTNAM ALAN R/IN
E7            1     PUTNAM ALBERT P III/IN
E8            5     PUTNAM ALLEN/IN
E9            2     PUTNAM ALLEN L/IN
E10           1     PUTNAM ALLEN LEWIS/IN
E11           1     PUTNAM ALLEN W/IN
E12           2     PUTNAM ALVIN A/IN

=> s e-e3
       2845142 E
         27154 E3
L2          77 E-E3
               (E(W)E3)

=> del 12
DELETE L2? (Y)/N:y

=> s e2-e3
            3 "PUTNAK J ROBERT"/IN
            6 "PUTNAK JOSEPH R"/IN
L2          9 ("PUTNAK J ROBERT"/IN OR "PUTNAK JOSEPH R"/IN)

=> s l1 not l1
L3          0 L1 NOT L1

=> e dubois doria r/in
E1            8     DUBOIS DONN ANTHONY/IN
E2            1     DUBOIS DONNA A/IN
E3            6 --> DUBOIS DORIA R/IN
E4            1     DUBOIS DORIS R/IN
E5            2     DUBOIS DOUG/IN
E6            1     DUBOIS DUNILAC DANIEL/IN
E7            5     DUBOIS DWIGHT/IN
E8            7     DUBOIS DWIGHT B/IN
E9            6     DUBOIS EDMUND H/IN
E10           4     DUBOIS EDWARD A/IN
E11           1     DUBOIS EDWARD F/IN
E12           1     DUBOIS EDWARD J/IN

=> s e3
L4            6 "DUBOIS DORIA R"/IN
```

```
L5          0 L4 NOT L1

=> file wpids
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    24.86      25.07

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    INDEX ENHANCEMENTS PLEASE VISIT:
http://www.scientific.thomson.com/cm/dwpienhancements <<<

=> e eckels k/in
E1            2     ECKELS J F/IN
E2            4     ECKELS J M/IN
E3            3 --> ECKELS K/IN
E4           11     ECKELS K H/IN
E5            4     ECKELS P/IN
E6           33     ECKELS P W/IN
E7            2     ECKELS R E/IN
E8            1     ECKELS S/IN
E9            1     ECKELS T L/IN
E10           1     ECKELSBACH K/IN
E11           1     ECKELSBERGER W/IN
E12           2     ECKELT B/IN

=> s e3 or e4
              3 "ECKELS K"/IN
             11 "ECKELS K H"/IN
L6           13 "ECKELS K"/IN OR "ECKELS K H"/IN

=> d 16,bib,1-13

L6   ANSWER 1 OF 13  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   2004-079843 [08]   WPIDS
CR   1991-361683 [49]; 1992-123683 [15]; 1993-117535 [14]; 2001-181861 [18]
DNC  C2004-032522
TI   New recombinant genetic construct adapted to encode a chimeric flaviviral
     genome, useful for producing vaccines for the dengue virus and other
     flaviviruses, including tick-borne encephalitis virus and Japanese B
     encephalitis virus.
DC   B04 D16
IN   BRAY, M; CHANOCK, R M; ECKELS, K H; LAI, C; MEN, R; PLETNEV, A G; ZHANG, Y
PA   (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC  1
PI   US 6676936      B1 20040113 (200408)*      66
ADT  US 6676936 B1 CIP of US 1990-610206 19901108, CIP of US 1991-761224
     19910919, CIP of WO 1992-US7916 19920918, CIP of US 1992-957075 19921006,
     Cont of US 1993-173190 19931223, Cont of US 1994-250802 19940527, US
     2000-643217 20000818
FDT  US 6676936 B1 Cont of US 6184024
PRAI US 1993-173190       19931223; US 1990-610206       19901108;
     US 1991-761224       19910919; WO 1992-US7916       19920918;
     US 1992-957075       19921006; US 1994-250802       19940527;
     US 2000-643217       20000818

L6   ANSWER 2 OF 13  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   2003-615945 [58]   WPIDS
DNN  N2003-490431       DNC C2003-168023
TI   Preparing microfluidized lysate preparation for immunizing a subject, by
     microfluidizing a slurry of Leishmania parasite through a chamber and
     disrupting the parasite with a sudden release of pressure.
DC   B04 D16 S03
```

J M
PA (BALL-I) BALLOU W R; (ECKE-I) ECKELS K H; (GROG-I) GROGL M; (MAGI-I) MAGILL A J; (ROWT-I) ROWTON E D; (STIT-I) STITELER J M; (USSA) US ARMY MEDICAL RES & MATERIAL COMMAND
CYC 98
PI US 2003072714 A1 20030417 (200358)* 11
WO 2003033533 A1 20030424 (200358)# EN
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
EP 1436325 A1 20040714 (200446)# EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
AU 2002211673 A1 20030428 (200461)#
ADT US 2003072714 A1 US 2001-975020 20011012; WO 2003033533 A1 WO 2001-US31894 20011012; EP 1436325 A1 EP 2001-979743 20011012, WO 2001-US31894 20011012; AU 2002211673 A1 WO 2001-US31894 20011012, AU 2002-211673 20011012
FDT EP 1436325 A1 Based on WO 2003033533; AU 2002211673 A1 Based on WO 2003033533
PRAI US 2001-975020 20011012; WO 2001-US31894 20011012;
EP 2001-979743 20011012; AU 2002-211673 20011012

L6 ANSWER 3 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2001-388924 [41] WPIDS
DNC C2001-118589
TI Dengue virus vaccine for immunizing and protecting humans against dengue fever.
DC B04 D16
IN DUBOIS, D R; **ECKELS, K**; PUTNAK, J R
PA (USSA) US SEC OF ARMY
CYC 1
PI US 6254873 B1 20010703 (200141)* 14
ADT US 6254873 B1 US 1995-423338 19950417
PRAI US 1995-423338 19950417

L6 ANSWER 4 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2001-225921 [23] WPIDS
DNC C2001-067381
TI New immunoassay for dengue virus antibodies comprises using a purified inactivated dengue virus.
DC B04 D16
IN CASSIDY, K; DUBOIS, D R; **ECKELS, K**; PUTNAK, J R
PA (USSA) US SEC OF ARMY
CYC 1
PI US 6190859 B1 20010220 (200123)* 14
ADT US 6190859 B1 US 1995-422458 19950417
PRAI US 1995-422458 19950417

L6 ANSWER 5 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2001-181861 [18] WPIDS
CR 1991-361683 [49]; 1992-123683 [15]; 1993-117535 [14]; 2004-079843 [08]
DNC C2001-054182
TI Chimeric virus, useful as a vaccine against dengue virus and other flaviviruses, comprises nucleic acids encoding a structural protein from one flavivirus and a nonstructural protein from another flavivirus.
DC B04 D16
IN BRAY, M; CHANOCK, R M; **ECKELS, K H**; LAI, C; MEN, R; PLETNEV, A G; ZHANG, Y
PA (USSH) US DEPT HEALTH & HUMAN SERVICES
CYC 1
PI US 6184024 B1 20010206 (200118)* 65
ADT US 6184024 B1 Cont of US 1988-218852 19880714, CIP of US 1990-610206 19901108, CIP of US 1991-761222 19910919, CIP of US 1991-761224 19910919, CIP of US 1992-957075 19921007, Cont of US 1993-173190 19931223, US 1994-250802 19940527
PRAI US 1993-173190 19931223; US 1988-218852 19880714;
US 1990-610206 19901108; US 1991-761222 19910919;
US 1991-761224 19910919; US 1992-957075 19921007;
US 1994-250802 19940527

L6 ANSWER 6 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2000-647205 [62] WPIDS
DNC C2000-195764
TI Immunogenic composition comprising attenuated dengue-4 virus useful as vaccines for stimulating immune system of individual to induce protection against dengue-4 virus serotype.

IN DUBOIS, D R; **ECKELS, K H**; HOKE, C H; INNIS, B L; PUTNAK, J R; VAUGHN, D W; VAUGHN, D
PA (REED-N) REED ARMY INST RES WALTER; (USSA) US SEC OF ARMY
CYC 83
PI WO 2000057910 A1 20001005 (200062)* EN 101
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 2000040404 A 20001016 (200106)
EP 1165129 A1 20020102 (200209) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
JP 2002540171 W 20021126 (200307) 95
US 6537557 B1 20030325 (200325)
ADT WO 2000057910 A1 WO 2000-US8277 20000324; AU 2000040404 A AU 2000-40404 20000324; EP 1165129 A1 EP 2000-919775 20000324, WO 2000-US8277 20000324; JP 2002540171 W JP 2000-607660 20000324, WO 2000-US8277 20000324; US 6537557 B1 Provisional US 1999-126318P 19990326, Provisional US 2000-182068P 20000211, US 2000-534726 20000324
FDT AU 2000040404 A Based on WO 2000057910; EP 1165129 A1 Based on WO 2000057910; JP 2002540171 W Based on WO 2000057910
PRAI US 2000-182068P 20000211; US 1999-126318P 19990326; US 2000-534726 20000324

L6 ANSWER 7 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2000-647204 [62] WPIDS
DNC C2000-195763
TI Immunogenic composition for stimulating dengue-2 virus specific immune response, comprises attenuated dengue-2 virus.
DC B04 D16
IN DUBOIS, D R; **ECKELS, K H**; HENCHAL, E A; HOKE, C H; INNIS, B L; KANESA-THASAN, N; PUTNAK, J R; VAUGHN, D; HENCHAI, E A
PA (REED-N) REED ARMY INST RES WALTER; (USSA) US SEC OF ARMY
CYC 83
PI WO 2000057909 A2 20001005 (200062)* EN 97
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 2000040402 A 20001016 (200106)
EP 1165128 A2 20020102 (200209) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
JP 2002540170 W 20021126 (200307) 95
US 6511667 B1 20030128 (200311)
ADT WO 2000057909 A2 WO 2000-US8275 20000324; AU 2000040402 A AU 2000-40402 20000324; EP 1165128 A2 EP 2000-919773 20000324, WO 2000-US8275 20000324; JP 2002540170 W JP 2000-607659 20000324, WO 2000-US8275 20000324; US 6511667 B1 Provisional US 1999-126319P 19990326, Provisional US 2000-182067P 20000211, US 2000-534725 20000324
FDT AU 2000040402 A Based on WO 2000057909; EP 1165128 A2 Based on WO 2000057909; JP 2002540170 W Based on WO 2000057909
PRAI US 2000-182067P 20000211; US 1999-126319P 19990326; US 2000-534725 20000324

L6 ANSWER 8 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2000-638318 [61] WPIDS
DNC C2000-192015
TI Immunogenic composition comprising attenuated dengue-1, -2, -3 or -4 virus useful as vaccines for stimulating immune system of individual to induce protection against all 4 dengue virus serotypes.
DC B04 D16
IN DUBOIS, D R; **ECKELS, K H**; HOKE, C H; INNIS, B L; NIRANJAN, K; PUTNAK, J R; WELLINGTON, S; **ECKELS, K**; KANESA-THASAN, N; PRTNAK, J R; KANESSA-THASAN, N
PA (REED-N) REED ARMY INST RES WALTER; (USSA) US SEC OF ARMY
CYC 83
PI WO 2000057907 A2 20001005 (200061)* EN 95
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 2000040382 A 20001016 (200106)

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT
RO SE SI
KR 2002008136 A 20020129 (200253)
CN 1351502 A 20020529 (200258)
JP 2002540168 W 20021126 (200307) 92
US 6638514 B1 20031028 (200372)
BR 2000010969 A 20031223 (200406)
MX 2001009683 A1 20030601 (200417)
AU 779280 B2 20050113 (200512)
MX 230950 B 20050930 (200617)
CN 1191092 C 20050302 (200634)
ADT WO 2000057907 A2 WO 2000-US8199 20000324; AU 2000040382 A AU 2000-40382 20000324; EP 1165127 A2 EP 2000-919748 20000324, WO 2000-US8199 20000324; KR 2002008136 A KR 2001-712302 20010926; CN 1351502 A CN 2000-807995 20000324; JP 2002540168 W JP 2000-607657 20000324, WO 2000-US8199 20000324; US 6638514 B1 Provisional US 1999-126313P 19990326, Provisional US 2000-181724P 20000211, US 2000-535117 20000324; BR 2000010969 A BR 2000-10969 20000324, WO 2000-US8199 20000324; MX 2001009683 A1 WO 2000-US8199 20000324, MX 2001-9683 20010926; AU 779280 B2 AU 2000-40382 20000324; MX 230950 B WO 2000-US8199 20000324, MX 2001-9683 20010926; CN 1191092 C CN 2000-807995 20000324
FDT AU 2000040382 A Based on WO 2000057907; EP 1165127 A2 Based on WO 2000057907; JP 2002540168 W Based on WO 2000057907; BR 2000010969 A Based on WO 2000057907; MX 2001009683 A1 Based on WO 2000057907; AU 779280 B2 Previous Publ. AU 2000040382, Based on WO 2000057907; MX 230950 B Based on WO 2000057907
PRAI US 2000-181724P 20000211; US 1999-126313P 19990326;
US 2000-535117 20000324

L6 ANSWER 9 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2000-611687 [58] WPIDS
DNC C2000-183093
TI Immunogenic composition containing attenuated dengue-1 virus, useful as a protective vaccine, inducing humoral and cellular responses.
DC B04 D16
IN DUBOIS, D R; **ECKELS, K H**; HOKE, C H; INNIS, B L; PUTNAK, J R; VAUGHN, D W
PA (REED-N) REED ARMY INST RES WALTER
CYC 83
PI WO 2000057908 A2 20001005 (200058)* EN 96
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 2000041792 A 20001016 (200106)
EP 1165131 A2 20020102 (200209) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
JP 2002540169 W 20021126 (200307) 94
ADT WO 2000057908 A2 WO 2000-US8201 20000324; AU 2000041792 A AU 2000-41792 20000324; EP 1165131 A2 EP 2000-921482 20000324, WO 2000-US8201 20000324; JP 2002540169 W JP 2000-607658 20000324, WO 2000-US8201 20000324
FDT AU 2000041792 A Based on WO 2000057908; EP 1165131 A2 Based on WO 2000057908; JP 2002540169 W Based on WO 2000057908
PRAI US 2000-182064P 20000211; US 1999-126317P 19990326

L6 ANSWER 10 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2000-611686 [58] WPIDS
DNC C2000-183092
TI Immunological compositions containing attenuated dengue-1 virus, useful for vaccinating against dengue-1 infections.
DC B04 D16
IN DUBOIS, D R; **ECKELS, K H**; HOKE, C H; INNIS, B L; PUTNAK, J R; VAUGHN, D
PA (REED-N) REED ARMY INST RES WALTER; (USSA) US SEC OF ARMY
CYC 83
PI WO 2000057904 A2 20001005 (200058)* EN 99
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 2000041783 A 20001016 (200106)
EP 1165130 A2 20020102 (200209) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
JP 2002540166 W 20021126 (200307) 95
US 6528065 B1 20030304 (200320)
ADT WO 2000057904 A2 WO 2000-US8134 20000324; AU 2000041783 A AU 2000-41783

JP 2002540166 W JP 2000-607654 20000324, WO 2000-US8134 20000324; US 6528065 B1 Provisional US 1999-126311P 19990326, Provisional US 2000-182063P 20000211, US 2000-535684 20000324

FDT AU 2000041783 A Based on WO 2000057904; EP 1165130 A2 Based on WO 2000057904; JP 2002540166 W Based on WO 2000057904

PRAI US 2000-182063P 20000211; US 1999-126311P 19990326; US 2000-535684 20000324

L6 ANSWER 11 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 2000-602361 [57] WPIDS

DNC C2000-180351

TI Replicating dengue virus strains to high titer growth in mammalian cells for use as a vaccine.

DC B04 D16

IN **ECKELS, K H**; INNIS, B L; PUTNAK, J R

PA (REED-N) REED ARMY INST RES WALTER; (USSA) US SEC OF ARMY

CYC 83

PI WO 2000058444 A2 20001005 (200057)* EN 49

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ TZ UG ZW

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW

AU 2000040403 A 20001016 (200106)

EP 1165756 A2 20020102 (200209) EN

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

JP 2002539821 W 20021126 (200307) 46

US 6613556 B1 20030902 (200359)

AU 776638 B2 20040916 (200479)

ADT WO 2000058444 A2 WO 2000-US8276 20000324; AU 2000040403 A AU 2000-40403 20000324; EP 1165756 A2 EP 2000-919774 20000324, WO 2000-US8276 20000324; JP 2002539821 W JP 2000-608725 20000324, WO 2000-US8276 20000324; US 6613556 B1 Provisional US 1999-126316P 19990326, Provisional US 2000-182065P 20000211, US 2000-534724 20000324; AU 776638 B2 AU 2000-40403 20000324

FDT AU 2000040403 A Based on WO 2000058444; EP 1165756 A2 Based on WO 2000058444; JP 2002539821 W Based on WO 2000058444; AU 776638 B2 Previous Publ. AU 2000040403, Based on WO 2000058444

PRAI US 2000-182065P 20000211; US 1999-126316P 19990326; US 2000-534724 20000324

L6 ANSWER 12 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN

Full Text

AN 1999-243619 [20] WPIDS

DNC C1999-071001

TI Attenuated Japanese encephalitis.

DC B04 D16

IN BINN, L N; CHUNG, Y J; DUBOIS, D R; **ECKELS, K H**; HONG, S P; INNIS, B; KIM, H S; KIM, S O; LEE, S H; MOON, S B; PUTNAK, J R; SHIN, Y C; SRIVASTAVA, A K; YOO, W D; SRIVASTAVA, A I; SRIBASTABA, A K; PUNIAK, J R

PA (CHEI-N) CHEIL JEDANG CORP; (REED-N) REED ARMY INST RES WALTER; (CHEI-N) CHEIL JEDANG CO; (CHEI-N) CHEIL FOODS & CHEM INC; (USSA) US SEC OF ARMY

CYC 83

PI WO 9911762 A1 19990311 (199920)* EN 34

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW

AU 9890047 A 19990322 (199931)

KR 99023955 A 19990325 (200024)

EP 1025209 A1 20000809 (200039) EN

R: BE CH DE DK ES FR GB IT LI NL

CN 1272879 A 20001108 (200114)

NZ 503522 A 20010629 (200140)

JP 2001514844 W 20010918 (200169) 33

US 6309650 B1 20011030 (200172)

AU 748730 B 20020613 (200251)

KR 314404 B 20021012 (200325)

CA 2301000 C 20030708 (200352) EN

CN 1142271 C 20040317 (200577)

EP 1604685 A2 20051214 (200582) EN

R: BE CH DE DK ES FR GB IT LI NL

ADT WO 9911762 A1 WO 1998-KR259 19980825; AU 9890047 A AU 1998-90047 19980825; KR 99023955 A KR 1998-35007 19980827; EP 1025209 A1 EP 1998-941885 19980825, WO 1998-KR259 19980825; CN 1272879 A CN 1998-809797 19980825; NZ 503522 A NZ 1998-503522 19980825, WO 1998-KR259 19980825; JP 2001514844 W WO 1998-KR259 19980825, JP 2000-508774 19980825; US 6309650 B1 WO

19980825; KR 314404 B KR 1998-35007 19980827; CA 2301000 C CA 1998-2301000 19980825, WO 1998-KR259 19980825; CN 1142271 C CN 1998-809797 19980825; EP 1604685 A2 Div ex EP 1998-941885 19980825, EP 2005-14317 19980825

FDT AU 9890047 A Based on WO 9911762; EP 1025209 A1 Based on WO 9911762; NZ 503522 A Based on WO 9911762; JP 2001514844 W Based on WO 9911762; US 6309650 B1 Based on WO 9911762; AU 748730 B Previous Publ. AU 9890047, Based on WO 9911762; KR 314404 B Previous Publ. KR 99023955; CA 2301000 C Based on WO 9911762; EP 1604685 A2 Div ex EP 1025209

PRAI KR 1997-42002 19970828; KR 1997-42001 19970828

L6 ANSWER 13 OF 13 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 1988-006984 [01] WPIDS
DNC C1988-003120
TI Hepatitis A virus variants - without foetal bovine serum dependence and/or with rapid passage capability.
DC B04 D16
IN BERMAN, S L; BINN, L N; DUBOIS, D R; **ECKELS, K H**
PA (USSA) US SEC OF ARMY
CYC 1
PI US 693 A0 19871013 (198801)* 17
ADT US 693 A0 US 1987-693 19870105
PRAI US 1987-693 19870105

=> e putnak j r/in
E1 1 PUTNA Y A/IN
E2 3 PUTNA Z/IN
E3 12 --> PUTNAK J R/IN
E4 2 PUTNAM A/IN
E5 1 PUTNAM A A/IN
E6 1 PUTNAM A M/IN
E7 1 PUTNAM A P/IN
E8 5 PUTNAM A R/IN
E9 1 PUTNAM A W/IN
E10 1 PUTNAM B/IN
E11 1 PUTNAM B C H/IN
E12 1 PUTNAM B D/IN

=> s e3
L7 12 "PUTNAK J R"/IN

=> s l7 not l6
L8 3 L7 NOT L6

=> d l8,bib,1-3

L8 ANSWER 1 OF 3 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2006-303402 [32] WPIDS
TI An attenuated Japanese encephalitis virus adapted to Vero cell and a Japanese encephalitis vaccine.
DC B04 D16
IN KIM, H; KIM, S; **PUTNAK, J R**; SRIVASTAVA, A K &; YOO, W
PA (CHEI-N) CHEIL JEDANG INC; (REED-N) REED ARMY INST RES WALTER
CYC 1
PI TW 228147 B1 20050221 (200632)*
ADT TW 228147 B1 TW 1999-102520 19990222
PRAI TW 1999-102520 19990222

L8 ANSWER 2 OF 3 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN 2003-636686 [60] WPIDS
DNC C2003-174077
TI Novel nucleic acid chimera comprising nucleic acids encoding structural protein from West Nile virus and non-structural proteins from wild-type strain of dengue virus useful for producing live West Nile virus vaccines.
DC B04 C06 D16
IN BLANEY, J E; CHANOCK, R M; MURPHY, B R; PLETNEV, A G; **PUTNAK, J R**; WHITEHEAD, S S
PA (USSH) US DEPT HEALTH & HUMAN SERVICES; (BLAN-I) BLANEY J E; (CHAN-I) CHANOCK R M; (MURP-I) MURPHY B R; (PLET-I) PLETNEV A G; (PUTN-I) PUTNAK J R; (WHIT-I) WHITEHEAD S S
CYC 103
PI WO 2003059384 A1 20030724 (200360)* EN 52
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW

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     EP 1467754      A1 20041020 (200469)  EN
         R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV
            MC MK NL PT RO SE SI SK TR
     US 2005100886   A1 20050512 (200532)
     JP 2005521384   W  20050721 (200549)       60
ADT  WO 2003059384 A1 WO 2003-US594 20030109; AU 2003216046 A1 AU 2003-216046
     20030109; EP 1467754 A1 EP 2003-729602 20030109, WO 2003-US594 20030109;
     US 2005100886 A1 Provisional US 2002-347281P 20020110, Cont of WO
     2003-US594 20030109, US 2004-871775 20040618; JP 2005521384 W JP
     2003-559545 20030109, WO 2003-US594 20030109
FDT  AU 2003216046 A1 Based on WO 2003059384; EP 1467754 A1 Based on WO
     2003059384; JP 2005521384 W Based on WO 2003059384
PRAI US 2002-347281P      20020110; US 2004-871775       20040618

L8   ANSWER 3 OF 3  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
AN   2000-593647 [56]   WPIDS
DNC  C2000-177270
TI   Method for the diagnosis of dengue virus comprising detectingbinding
     between patient serum and recombinant proteins, useful for diagnosing
     dengue fever, dengue hemorrhagic fever, and dengue shock syndrome.
DC   B04 D16
IN   HOKE, C H; PUTNAK, J R; SRIVASTAVA, A K; WARREN, R L
PA   (USSA) US SEC OF ARMY
CYC  1
PI   US 6117640      A  20000912 (200056)*      16
ADT  US 6117640 A US 1995-433263 19950502
PRAI US 1995-433263       19950502


=> e dubois d r/in
E1           1     DUBOIS D L/IN
E2           6     DUBOIS D M/IN
E3          29 --> DUBOIS D R/IN
E4           1     DUBOIS D S/IN
E5          20     DUBOIS D W/IN
E6           1     DUBOIS DUNILAC D/IN
E7          29     DUBOIS E/IN
E8           6     DUBOIS E A/IN
E9           2     DUBOIS E F/IN
E10          5     DUBOIS E H/IN
E11          2     DUBOIS E J/IN
E12          1     DUBOIS E W/IN

=> s e3
L9          29 "DUBOIS D R"/IN

=> d his

     (FILE 'HOME' ENTERED AT 11:02:05 ON 27 JUN 2006)

     FILE 'USPATFULL' ENTERED AT 11:02:14 ON 27 JUN 2006
                E ECKELS KENNETH/IN
L1           11 S E3-E4
                E PUTNAK JOSEPH R/IN
L2            9 S E2-E3
L3            0 S L1 NOT L1
                E DUBOIS DORIA R/IN
L4            6 S E3
L5            0 S L4 NOT L1

     FILE 'WPIDS' ENTERED AT 11:03:30 ON 27 JUN 2006
                E ECKELS K/IN
L6           13 S E3 OR E4
                E PUTNAK J R/IN
L7           12 S E3
L8            3 S L7 NOT L6
                E DUBOIS D R/IN
L9           29 S E3

=> s l9 not (l6 or l8)
L10          20 L9 NOT (L6 OR L8)

=> s l10 and (den? or flavivir?)
        459286 DEN?
           486 FLAVIVIR?
L11           1 L10 AND (DEN? OR FLAVIVIR?)

=> d l11,bib

L11  ANSWER 1 OF 1  WPIDS  COPYRIGHT 2006 THE THOMSON CORP on STN
Full Text
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CR 1996-232107 [24]; 1997-291302 [27]; 1998-065318 [07]; 1998-065319 [07]; 1998-498213 [43]; 1999-430703 [36]; 2000-052268 [04]; 2001-241577 [25]; 2002-706398 [76]; 2003-103049 [09]
DNN N1997-251843
TI Bias plasma annealing method for thin film deposition on semiconductor substrate - involves performing nitride titanium film deposition on substrate applied with bias to make ion collide with nitride titanium film.
DC U11
IN CHANG, M; DANEK, M; **DUBOIS, D R**; ENGLHARDT, E; KAO, Y; LIAO, M; MORRISON, A F
PA (MATE-N) APPLIED MATERIALS INC
CYC 2
PI JP 09115917 A 19970502 (199728)* 15
US 2004099215 A1 20040527 (200435)
ADT JP 09115917 A JP 1996-178103 19960708; US 2004099215 A1 Cont of US 1995-498990 19950706, Cont of US 1995-567461 19951205, Cont of US 1996-677185 19960709, Cont of US 1996-677218 19960709, Cont of US 1996-680913 19960712, Cont of US 1997-808246 19970228, US 2003-716096 20031118
FDT US 2004099215 A1 Cont of US 6155198, Cont of US 6699530
PRAI US 1995-498990 19950706; US 1995-567461 19951205;
US 1996-677185 19960709; US 1996-677218 19960709;
US 1996-680913 19960712; US 1997-808246 19970228;
US 2003-716096 20031118

=> file medline
COST IN U.S. DOLLARS SINCE FILE ENTRY TOTAL SESSION
FULL ESTIMATED COST 79.19 104.26

FILE 'MEDLINE' ENTERED AT 11:05:22 ON 27 JUN 2006

FILE LAST UPDATED: 24 JUN 2006 (20060624/UP). FILE COVERS 1950 TO DATE.

On December 11, 2005, the 2006 MeSH terms were loaded.

The MEDLINE reload for 2006 is now (26 Feb.) available. For details on the 2006 reload, enter HELP RLOAD at an arrow prompt (=>). See also:

http://www.nlm.nih.gov/mesh/
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_med_data_changes.html
http://www.nlm.nih.gov/pubs/techbull/nd05/nd05_2006_MeSH.html

OLDMEDLINE is covered back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2006 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> e eckels k/au
E1 1 ECKELS J/AU
E2 1 ECKELS J A/AU
E3 1 --> ECKELS K/AU
E4 55 ECKELS K H/AU
E5 8 ECKELS KENNETH H/AU
E6 8 ECKELS R/AU
E7 5 ECKELS T/AU
E8 1 ECKELS T J/AU
E9 4 ECKELT A/AU
E10 1 ECKELT BIRGIT/AU
E11 1 ECKELT D/AU
E12 1 ECKELT G/AU

=> s e3-e5
1 "ECKELS K"/AU
55 "ECKELS K H"/AU
8 "ECKELS KENNETH H"/AU
L12 64 ("ECKELS K"/AU OR "ECKELS K H"/AU OR "ECKELS KENNETH H"/AU)

=> s l12 and (flavivir? or den?)
3739 FLAVIVIR?
904277 DEN?
L13 50 L12 AND (FLAVIVIR? OR DEN?)

=> s l13 and vaccin?
167376 VACCIN?

```
=> s l14 and (multivalent)
          2699 MULTIVALENT
L15          1 L14 AND (MULTIVALENT)

=> d l15,cbib,ab

L15  ANSWER 1 OF 1     MEDLINE on STN
93381798.     PubMed ID: 8371350.    Dengue virus-specific human CD4+
     T-lymphocyte responses in a recipient of an experimental live-attenuated
     dengue virus type 1 vaccine: bulk culture proliferation, clonal
     analysis, and precursor frequency determination. Green S; Kurane I;
     Edelman R; Tacket C O; Eckels K H; Vaughn D W; Hoke C H Jr; Ennis F A.
     (Department of Medicine, University of Massachusetts Medical Center,
     Worcester 01655. ) Journal of virology, (1993 Oct) Vol. 67, No. 10, pp.
     5962-7.  Journal code: 0113724. ISSN: 0022-538X. Pub. country: United
     States. Language: English.
AB   We analyzed the CD4+ T-lymphocyte responses to dengue, West Nile, and
     yellow fever viruses 4 months after immunization of a volunteer with an
     experimental live-attenuated dengue virus type 1 vaccine (DEN-1
     45AZ5). We examined bulk culture proliferation to noninfectious antigens,
     determined the precursor frequency of specific CD4+ T cells by limiting
     dilution, and established and analyzed CD4+ T-cell clones. Bulk culture
     proliferation was predominantly dengue virus type 1 specific with a
     lesser degree of cross-reactive responses to other dengue virus
     serotypes, West Nile virus, and yellow fever virus. Precursor frequency
     determination by limiting dilution in the presence of noninfectious
     dengue virus antigens revealed a frequency of antigen-reactive cells of
     1 in 1,686 peripheral blood mononuclear cells (PBMC) for dengue virus
     type 1, 1 in 9,870 PBMC for dengue virus type 3, 1 in 14,053 PBMC for
     dengue virus type 2, and 1 in 17,690 PBMC for dengue virus type 4.
     Seventeen CD4+ T-cell clones were then established by using infectious
     dengue virus type 1 as antigen. Two patterns of dengue virus
     specificity were found in these clones. Thirteen clones were dengue
     virus type 1 specific, and four clones recognized both dengue virus
     types 1 and 3. Analysis of human leukocyte antigen (HLA) restriction
     revealed that five clones are HLA-DRw52 restricted, one clone is HLA-DP3
     restricted, and one clone is HLA-DP4 restricted. These results indicate
     that in this individual, the CD4+ T-lymphocyte responses to immunization
     with live-attenuated dengue virus type 1 vaccine are predominantly
     serotype specific and suggest that a multivalent vaccine may be
     necessary to elicit strong serotype-cross-reactive CD4+ T-lymphocyte
     responses in such individuals.


=> d his

     (FILE 'HOME' ENTERED AT 11:02:05 ON 27 JUN 2006)

     FILE 'USPATFULL' ENTERED AT 11:02:14 ON 27 JUN 2006
                E ECKELS KENNETH/IN
L1           11 S E3-E4
                E PUTNAK JOSEPH R/IN
L2            9 S E2-E3
L3            0 S L1 NOT L1
                E DUBOIS DORIA R/IN
L4            6 S E3
L5            0 S L4 NOT L1

     FILE 'WPIDS' ENTERED AT 11:03:30 ON 27 JUN 2006
                E ECKELS K/IN
L6           13 S E3 OR E4
                E PUTNAK J R/IN
L7           12 S E3
L8            3 S L7 NOT L6
                E DUBOIS D R/IN
L9           29 S E3
L10          20 S L9 NOT (L6 OR L8)
L11           1 S L10 AND (DEN? OR FLAVIVIR?)

     FILE 'MEDLINE' ENTERED AT 11:05:22 ON 27 JUN 2006
                E ECKELS K/AU
L12          64 S E3-E5
L13          50 S L12 AND (FLAVIVIR? OR DEN?)
L14          42 S L13 AND VACCIN?
L15           1 S L14 AND (MULTIVALENT)

=> s l14 not l15
L16         41 L14 NOT L15

=> d l16,cbib,ab,1-41
```

2006272777. PubMed ID: 16703509. Protection of Rhesus monkeys against **dengue** virus challenge after tetravalent live attenuated **dengue** virus **vaccination**. Sun Wellington; Nisalak Ananda; Gettayacamin Montip; **Eckels Kenneth H**; Putnak J Robert; Vaughn David W; Innis Bruce L; Thomas Stephen J; Endy Timothy P. (Department of Virus Diseases, Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910-7500, USA.. wellinton.sun@us.army.mil) . The Journal of infectious diseases, (2006 Jun 15) Vol. 193, No. 12, pp. 1658-65. Electronic Publication: 2006-05-09. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB Rhesus monkeys develop viremia after **dengue** virus (**DENV**) inoculation and have been used as an animal model to study **DENV** infection and **DENV vaccine** candidates. We evaluated, in this model, the protective efficacy of a live attenuated tetravalent **DENV vaccine** (TDV) candidate against parenteral challenge with parental near-wild-type **DENV** strains. Twenty monkeys were **vaccinated** with TDV at 0 and 1 month, and 20 unvaccinated monkeys served as controls. **Vaccinated** animals and their controls were inoculated with 10(3)-10(4) pfu of challenge virus 4.5 months after the second **vaccination**. Primary **vaccination** resulted in 95%, 100%, 70%, and 15% seroconversion to **DENV** serotypes 1, 2, 3, and 4 (**DENV**-1, -2, -3, and -4), respectively. After the second **vaccination**, the seropositivity rates were 100%, 100%, 90%, and 70%, respectively. **Vaccination** with TDV resulted in complete protection against viremia from **DENV**-2 challenge and in 80%, 80%, and 50% protection against challenge with **DENV**-1, -3, and -4, respectively. Our results suggest that the TDV can elicit protective immunity against all 4 **DENV** serotypes. Interference among the 4 **vaccine** viruses may have resulted in decreased antibody responses to **DENV**-3 and -4, which would require reformulation or dose optimization to minimize this interference during testing of the **vaccine** in humans.

L16 ANSWER 2 OF 41 MEDLINE on STN

2005354017. PubMed ID: 16005749. An evaluation of **dengue** type-2 inactivated, recombinant subunit, and live-attenuated **vaccine** candidates in the rhesus macaque model. Robert Putnak J; Coller Beth-Ann; Voss Gerald; Vaughn David W; Clements David; Peters Iain; Bignami Gary; Houng Hou-Shu; Chen Robert C-M; Barvir David A; Seriwatana Jitvimol; Cayphas Sylvie; Garcon Nathalie; Gheysen Dirk; Kanesa-Thasan Niranjan; McDonell Mike; Humphreys Tom; **Eckels Kenneth H**; Prieels Jean-Paul; Innis Bruce L. (Walter Reed Army Institute of Research, Division of Communicable Diseases and Immunology, 503 Robert Grant Avenue, Silver Spring, MD 20910, USA.. robert.putnak@na.amedd.army.mil) . Vaccine, (2005 Aug 15) Vol. 23, No. 35, pp. 4442-52. Journal code: 8406899. ISSN: 0264-410X. Pub. country: Netherlands. Language: English.

AB The safety, immunogenicity, and protective efficacy of two non-replicating antigen-based **vaccines** and one live-attenuated virus (LAV) **vaccine** for **dengue** type-2 (**dengue**-2) virus were evaluated in the rhesus macaque model. The non-replicating **vaccines** consisted of whole, purified inactivated virus (PIV) and a recombinant subunit protein containing the amino-(N)-terminal 80% of envelope protein (r80E), each formulated with one of five different adjuvants. Each formulation was administered to three animals on a 0, 3-month schedule. Following the primary immunizations, 37 of 39 animals demonstrated **dengue**-2 virus neutralizing antibodies. After the booster immunizations all animals had **dengue** neutralizing antibodies with peak titers ranging from 1:100 to 1:9700. The highest neutralizing antibody titers were observed in the groups that received r80E antigen formulated with AS04, AS05, or AS08 adjuvant, and PIV formulated with AS05 or AS08 adjuvant. These newer adjuvants are based on alum, fraction QS-21 of saponin, and monophosphoryl lipid A (MPL). Protection was evaluated by **dengue**-2 virus challenge 2 months after the booster by the measurement of circulating virus (viremia) and post-challenge immune responses. Several groups exhibited nearly complete protection against viremia by bioassay, although there was evidence for challenge virus replication by Taqmantrade mark and immunological assays. None of the **vaccines** conferred sterile immunity.

L16 ANSWER 3 OF 41 MEDLINE on STN

2004054861. PubMed ID: 14756126. Progress in development of a live-attenuated, tetravalent **dengue** virus **vaccine** by the United States Army Medical Research and Materiel Command. Innis Bruce L; **Eckels Kenneth H**. The American journal of tropical medicine and hygiene, (2003 Dec) Vol. 69, No. 6 Suppl, pp. 1-4. Ref: 32. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

L16 ANSWER 4 OF 41 MEDLINE on STN

2004040706. PubMed ID: 14740955. Phase I trial of 16 formulations of a tetravalent live-attenuated **dengue vaccine**. Edelman Robert; Wasserman Steven S; Bodison Sacared A; Putnak Robert J; **Eckels Kenneth H**; Tang Douglas; Kanesa-Thasan Niranjan; Vaughn David W; Innis Bruce L; Sun Wellington. (Department of Medicine and the Center for Vaccine Development, University of Maryland School of Medicine, Baltimore,

journal of tropical medicine and hygiene, (2003 Dec) Vol. 69, No. 6 Suppl, pp. 48-60. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB Laboratory-attenuated strains of each of the four **dengue** serotypes previously tested as monovalent **vaccines** in volunteers were combined and tested for immunogenicity, safety, and reactogenicity in 16 dosage combinations. Tetravalent **vaccines** made using combinations of high (10(5-6) plaque-forming units [PFU]/dose) or low (10(3.5-4.5) PFU/dose) dosage formulations of each of the four viruses were inoculated in 64 **flavivirus** non-immune adult volunteers to determine which, if any, formulation raised neutralizing antibodies in at least 75% of volunteers to at least three of four **dengue** serotypes following one or two inoculations. Such formulations, if safe and sufficiently non-reactogenic, would be considered for an expanded Phase II trial in the future. Formulations 1-15 were each inoculated into three or four volunteers (total = 54) on days 0 and 28. Formulation 16 was tested in 10 volunteers, five volunteers inoculated on days 0 and 30, one volunteer on days 0 and 120, and four volunteers on days 0, 30, and 120. Blood was drawn for serologic assays immediately before and one month after each **vaccination**, and for viremia assay on day 10 after each **vaccination**. The 16 formulations were safe, but variably reactogenic after the first **vaccination**, and nearly non-reactogenic after the second and third **vaccinations**. Reactogenicity was positively correlated with immunogenicity. Similar proportions of volunteers seroconverted to **dengue**-1 (69%), **dengue**-2 (78%), and **dengue**-3 (69%), but significantly fewer volunteers seroconverted to **dengue**-4 (38%). The geometric mean 50% plaque reduction neutralization test titers in persons who seroconverted were significantly higher to **dengue**-1 (1:94) than to **dengue**-2 (1:15), **dengue**-3 (1:10), and **dengue**-4 (1:2). Seven formulations met the serologic criteria required for an expanded trial, and three of these were sufficiently attenuated clinically to justify further testing.

L16 ANSWER 5 OF 41 MEDLINE on STN

2004040703. PubMed ID: 14740952. **Vaccination** of human volunteers with monovalent and tetravalent live-attenuated **dengue vaccine** candidates. Sun Wellington; Edelman Robert; Kanesa-Thasan Niranjan; **Eckels Kenneth H**; Putnak J Robert; King Alan D; Houng Huo-Shu; Tang Douglas; Scherer John M; Hoke Charles H Jr; Innis Bruce L. (Department of Virus Diseases, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910, USA.. wellington.sun@na.amedd.army.mil) . The American journal of tropical medicine and hygiene, (2003 Dec) Vol. 69, No. 6 Suppl, pp. 24-31. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB Four serotypes of monovalent live attenuated **dengue** virus **vaccine** candidates were tested for reactogenicity and immunogenicity in 49 **flavivirus** non-immune adult human volunteers. The four monovalent candidates were then combined into a tetravalent formulation and given to another 10 volunteers. Neutralizing antibody seroconversion rates after a single-dose monovalent **vaccination** ranged from 53% to 100%. Solicited reactogenicity was scored by each volunteer. A composite index, the Reactogenicity Index, was derived by these self-reported scores. Reactogenicity differed among the four serotype candidates with serotype-1 associated with the most **vaccine** related side effects. A second dose of monovalent **vaccines** at either 30 days or 90 days was much less reactogenic but did not significantly increase seroconversion rates. Seroconversion rates in the 10 volunteers who received a single dose of tetravalent **vaccine** ranged from 30% to 70% among the four serotypes. Similar to the monovalent **vaccines**, a second dose of the tetravalent **vaccine** at one month was less reactogenic and did not increase seroconversion. A third dose of the tetravalent **vaccine** at four months resulted in three of four volunteers with trivalent or tetravalent high-titer neutralizing antibody responses.

L16 ANSWER 6 OF 41 MEDLINE on STN

2004040702. PubMed ID: 14740951. Phase 1 studies of Walter Reed Army Institute of Research candidate attenuated **dengue vaccines**: selection of safe and immunogenic monovalent **vaccines**. Kanesa-Thasan N; Edelman R; Tacket C O; Wasserman S S; Vaughn D W; Coster T S; Kim-Ahn G J; Dubois D R; Putnak J R; King A; Summers P L; Innis B L; **Eckels K H**; Hoke C H Jr. (Walter Reed Army Institute of Research, Washington, District of Columbia, USA.. niranjan.kanesa-thasan@det.amedd.army.mil) . The American journal of tropical medicine and hygiene, (2003 Dec) Vol. 69, No. 6 Suppl, pp. 17-23. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB We describe the results of initial safety testing of 10 live-attenuated **dengue** virus (**DENV**) **vaccine** candidates modified by serial passage in primary dog kidney (PDK) cells at the Walter Reed Army Institute of Research. The Phase 1 studies, conducted in 65 volunteers, were designed to select an attenuated **vaccine** candidate for each **DENV** serotype. No recipient of the **DENV** candidate **vaccines** sustained serious injury or required treatment. Three **vaccine** candidates were associated with

their withdrawal from further clinical development. Increasing PDK cell passage of **DENV**-1, **DENV**-2, and **DENV**-3 candidate **vaccines** increased attenuation for volunteers, yet also decreased infectivity and immunogenicity. This effect was less clear for **DENV**-4 candidate **vaccines** following 15 and 20 PDK cell passages. Only one passage level each of the tested **DENV**-2, -3, and -4 **vaccine** candidates was judged acceptably reactogenic and suitable for expanded clinical study. Subsequent studies with more recipients will further establish safety and immunogenicity of the four selected **vaccine** candidates: **DENV**-1 45AZ5 PDK 20, **DENV**-2 S16803 PDK 50, **DENV**-3 CH53489 PDK 20, and **DENV**-4 341750 PDK 20.

L16 ANSWER 7 OF 41 MEDLINE on STN

2004040701. PubMed ID: 14740950. Modification of **dengue** virus strains by passage in primary dog kidney cells: preparation of candidate **vaccines** and immunization of monkeys. **Eckels Kenneth H**; Dubois Doria R; Putnak Robert; Vaughn David W; Innis Bruce L; Henchal Erik A; Hoke Charles H Jr. (Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910, USA.) The American journal of tropical medicine and hygiene, (2003 Dec) Vol. 69, No. 6 Suppl, pp. 12-6. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB **Dengue** (**DENV**) virus strains for each of the four **DENV** serotypes were modified by passage in primary dog kidney (PDK) cell cultures with final manufacture of **vaccine** lots in fetal rhesus monkey diploid cell cultures. "Strain sets" consisting of serially-passaged **DENV** were inoculated in rhesus monkeys along with unmodified parent viruses for each strain. **Vaccine** candidates were compared with unmodified parent viruses by measuring viremia and immune responses. All except one **DENV**-1 strain demonstrated reduced infection in monkeys after PDK cell passage. A **DENV**-3 strain lost all monkey infectivity after PDK cell passage. Twelve **vaccine** candidates were selected for Phase 1 human trials through this selection process.

L16 ANSWER 8 OF 41 MEDLINE on STN

2004017651. PubMed ID: 14714438. Formalin-inactivated whole virus and recombinant subunit **flavivirus vaccines**. **Eckels Kenneth H**; Putnak Robert. (Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Silver Spring, Maryland 20910, USA.) Advances in virus research, (2003) Vol. 61, pp. 395-418. Ref: 84. Journal code: 0370441. ISSN: 0065-3527. Pub. country: United States. Language: English.

AB The **Flaviviridae** is a family of arthropod-borne, enveloped, RNA viruses that contain important human pathogens such as yellow fever (YF), Japanese encephalitis (JE), tick-borne encephalitis (TBE), West Nile (WN), and the **dengue** (**DEN**) viruses. **Vaccination** is the most effective means of disease prevention for these viral infections. A live-attenuated **vaccine** for YF, and inactivated **vaccines** for JE and TBE have significantly reduced the incidence of disease for these viruses, while licensed **vaccines** for **DEN** and WN are still lacking despite a significant disease burden associated with these infections. This review focuses on inactivated and recombinant subunit **vaccines** (non-replicating protein **vaccines**) in various stages of laboratory development and human testing. A purified, inactivated **vaccine** (PIV) candidate for **DEN** will soon be evaluated in a phase 1 clinical trial, and a second-generation JE PIV produced using similar technology has advanced to phase 2/3 trials. The inactivated TBE **vaccine** used successfully in Europe for almost 30 years continues to be improved by additional purification, new stabilizers, an adjuvant, and better immunization schedules. The recent development of an inactivated WN **vaccine** for domestic animals demonstrates the possibility of producing a similar **vaccine** for human use. Advances in **flavivirus** gene expression technology have led to the production of several recombinant subunit antigen **vaccine** candidates in a variety of expression systems. Some of these **vaccines** have shown sufficient promise in animal models to be considered as candidates for evaluation in clinical trials. Feasibility of non-replicating **flavivirus vaccines** has been clearly demonstrated and further development is now warranted.

L16 ANSWER 9 OF 41 MEDLINE on STN

1998161701. PubMed ID: 9502595. Evaluation of recombinant **dengue** viral envelope B domain protein antigens for the detection of **dengue** complex-specific antibodies. Simmons M; Porter K R; Escamilla J; Graham R; Watts D M; **Eckels K H**; Hayes C G. (U.S. Naval Medical Research Institute, Bethesda, Maryland 20889-5607, USA.) The American journal of tropical medicine and hygiene, (1998 Feb) Vol. 58, No. 2, pp. 144-51. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB To increase the specificity of **dengue** (**DEN**) diagnosis based on antibody detection, we have evaluated recombinant proteins as antigens that incorporate most of the B domain of the **DEN** virus envelope protein fused to the trpE protein of Escherichia coli (trpE-**DEN**). A pooled antigen consisting of trpE-**DEN** proteins representing all four serotypes

IgM antibody. This assay was compared with a standard IgG indirect ELISA and an IgM-capture ELISA using **DEN** virus-infected cell culture pooled antigens. The results indicated that the trpE-**DEN** antigens and the cell culture antigens were equally sensitive for detecting IgM and IgG antibodies in convalescent sera from Peru and Indonesia representing virus isolation-confirmed primary and secondary **DEN** infections, respectively. Fourteen day postinfection IgG antibody-positive sera obtained from individuals infected with **DEN**-1 virus who had been **vaccinated** with other **flaviviruses** were more strongly reactive with the cell culture antigen than with the recombinant antigen, but by day 21 postinfection, a strong antibody response to the trpE-**DEN** antigens was present. These results suggested that the early antibody response was directed predominantly towards shared **flavivirus** group antigens that were not detected with the trpE-**DEN** antigens. Comparison of the trpE-**DEN**-1 recombinant antigen with a **DEN**-1 virus-infected cell lysate antigen for the detection of IgG antibody in sera from a cohort of 55 individuals from Peru who seroconverted over a one-year period indicated greater specificity for the recombinant antigens. Also, sera from individuals with no known **DEN** infections that had been sequentially **vaccinated** with yellow fever and Japanese encephalitis reacted with the **DEN** virus cell culture antigen in the IgG ELISA, but did not react with the trpE-**DEN** pooled antigens. Similarly, YF IgM antibody positive samples that showed cross-reactivity with the **DEN** virus cell culture antigens, did not react with the trpE-**DEN** pooled antigens. These results indicated that the trpE-**DEN** pooled antigen provided a more specific diagnosis of **dengue** infections than **DEN** virus-infected cell culture antigen and avoided the biohazards associated with handling live virus during the preparation of diagnostic reagents. The trpE-**DEN** pooled antigen should permit a better approach to distinguish between past **DEN** and other **flavivirus** infections in epidemiologic surveys, and also increase the specificity of serologic diagnosis of acute **DEN** infections.

L16 ANSWER 10 OF 41 MEDLINE on STN

1998030041. PubMed ID: 9363590. T cell activation in vivo by **dengue** virus infection. Kurane I; Innis B L; Hoke C H Jr; **Eckels K H**; Meager A; Janus J; Ennis F A. (Department of Medicine, University of Massachusetts Medical Center, Worcester 01655, USA.) Journal of clinical & laboratory immunology, (1995) Vol. 46, No. 1, pp. 35-40. Journal code: 7808987. ISSN: 0141-2760. Pub. country: SCOTLAND: United Kingdom. Language: English.

AB It is accepted that T cells play a critical role during virus infections; however, T cell responses in vivo in acute stage of virus infection are not understood. We examined T cell activation in vivo in two volunteers who developed **dengue** fever in response to **vaccination** with a candidate live **dengue vaccine**. Serial plasma collected from the volunteers from day 0 (before infection) to day 17 after infection were examined for levels of soluble interleukin-2 receptor (sIL-2R), soluble CD4 (sCD4), soluble CD8 (sCD8), interleukin-2 (IL-2) and interferon gamma (INF gamma). Elevation of the levels of sIL-2R, IFN gamma, sCD4 and IL-2 became obvious during the period of viremia and was followed by a later increase in the level of sCD8. The levels of IFN gamma and sIL-2R declined after the end of the period of viremia. These results indicate that i. T cells are activated in vivo by **dengue** virus infection ii. activation of CD4+ T cells occurs during the period of viremia iii. activation of CD8+ T cells follows CD4+ T cell activation. These results suggest that activation of T cells in vivo may contribute to controlling acute **dengue** virus infections.

L16 ANSWER 11 OF 41 MEDLINE on STN

97437483. PubMed ID: 9292016. Molecular analysis of **dengue** virus attenuation after serial passage in primary dog kidney cells. Puri B; Nelson W M; Henchal E A; Hoke C H; **Eckels K H**; Dubois D R; Porter K R; Hayes C G. (Infectious Diseases Department, Naval Medical Research Institute, Bethesda, Maryland 20889-5607, USA.. PURI@MAIL2.NMRI.NNMC.NAVY.MIL) . The Journal of general virology, (1997 Sep) Vol. 78 (Pt 9), pp. 2287-91. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The complete nucleotide sequences of the genomes of **dengue**-1 virus virulent 45AZ5 PDK-0 and attenuated **vaccine** candidate strain 45AZ5 PDK-27 have been determined and compared with the **dengue**-1 virus Western Pacific (West Pac) 74 parent strain from which 45AZ5 PDK-0 was derived. Twenty-five (0.23%) nucleotide and 10 (0.29%) amino acid substitutions occurred between parent strain **dengue**-1 virus West Pac 74 and virulent strain 45AZ5 PDK-0, which was derived from the parent by serial passage in diploid foetal rhesus lung (FRhL-2) and mutagenized with 5-azacytidine. These substitutions were preserved in the 45AZ5 PDK-27 **vaccine**. 45AZ5 PDK-0 and PDK-27 strains, which differ by 27 passages in primary dog kidney (PDK) cells, show 25 (0.23%) nucleotide and 11 (0.32%) amino acid divergences. These comparative studies suggest that the changes which occurred between the West Pac 74 and 45AZ5 PDK-0 strains may alter the biological properties of the virus but may not be important for attenuation. Important nucleotide base changes responsible for

L16 ANSWER 12 OF 41 MEDLINE on STN
97094118. PubMed ID: 8940206. Development of a purified, inactivated, **dengue**-2 virus **vaccine** prototype in Vero cells: immunogenicity and protection in mice and rhesus monkeys. Putnak R; Barvir D A; Burrous J M; Dubois D R; D'Andrea V M; Hoke C H; Sadoff J C; **Eckels K H**. (Walter Reed Army Institute of Research, Division of Communicable Diseases and Immunology, Washington, DC 20307-5100, USA.) The Journal of infectious diseases, (1996 Dec) Vol. 174, No. 6, pp. 1176-84. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB The feasibility of a purified, inactivated **dengue** (**DEN**) **vaccine** made in Vero cells was explored. A **DEN**-2 virus candidate was chosen for production of a monotypic, purified, inactivated **vaccine** (PIV). Virus was harvested from roller bottle culture supernatants, concentrated, and purified on sucrose gradients. The purified virus was inactivated with 0.05% formalin at 22 degrees C. After inactivation, the virus retained its antigenicity and was immunogenic in mice and rhesus monkeys, in which it elicited high titers of **DEN**-2 virus-neutralizing antibody. Mice were completely protected against challenge with live, virulent virus after receiving two 0.15-microg doses of PIV. Monkeys **vaccinated** with three doses ranging as low as 0.25 microg demonstrated complete absence or a significant reduction in the number of days of viremia after challenge with homologous virus. These results warrant further testing and development of PIVs for other **DEN** virus serotypes.

L16 ANSWER 13 OF 41 MEDLINE on STN
95201101. PubMed ID: 7893886. Quantitative relationship between oral temperature and severity of illness following inoculation with candidate attenuated **dengue** virus **vaccines**. Mackowiak P A; Wasserman S S; Tacket C O; Vaughn D W; **Eckels K H**; Dubois D R; Hoke C H; Edelman R. (Department of Veterans Affairs Medical Center, Baltimore, Maryland 21201.) Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, (1994 Nov) Vol. 19, No. 5, pp. 948-50. Journal code: 9203213. ISSN: 1058-4838. Pub. country: United States. Language: English.

AB The relationship between oral temperature and other parameters of illness was examined in 51 adult volunteers who were inoculated experimentally with partially attenuated candidate **dengue** virus **vaccines**. In subjects who developed clinical illness, the peak illness temperature, mean illness temperature, and peak 6:00 A.M. illness temperature all correlated positively with the total number of signs and symptoms other than fever and with a fall in the white blood cell count (the latter was the only laboratory abnormality significantly associated with clinical illness [P = .02]). Of these factors, the peak 6:00 A.M. oral temperature exhibited the strongest correlations with the two parameters used to estimate severity of illness (rxy = .58 and P < .01 for signs and symptoms; rxy = .37 and P = .01 for fall in white blood cell count).

L16 ANSWER 14 OF 41 MEDLINE on STN
95088425. PubMed ID: 7995984. A live attenuated **dengue**-1 **vaccine** candidate (45AZ5) passaged in primary dog kidney cell culture is attenuated and immunogenic for humans. Edelman R; Tacket C O; Wasserman S S; Vaughn D W; **Eckels K H**; Dubois D R; Summers P L; Hoke C H. (Department of Medicine, University of Maryland School of Medicine, Baltimore 21201.) The Journal of infectious diseases, (1994 Dec) Vol. 170, No. 6, pp. 1448-55. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB A **dengue**-1 candidate **vaccine** (45AZ5), previously found to be underattenuated in 2 volunteers, was further attenuated by passage in primary dog kidney (PDK) cell cultures. New candidate **vaccines** prepared from three levels of PDK-passaged virus, PDK-10, PDK-20, and PDK-27, were each injected into 9 or 10 volunteers. There was a significant, progressive decline in viremia, clinical illness, and hematologic changes from low to high PDK cell passage level. PDK-20 infected all 10 **vaccinees** and induced viremia in 5, transient fever in 3, symptoms that resulted in curtailed activities for < or = 1 day in 4, and neutralizing antibody in all 10, which persisted for > or = 1 year in 5 of 8 **vaccinees** tested. Progressive passage in PDK cell culture progressively attenuates **vaccine** candidate strain 45AZ5 for humans. Because passage level PDK-20 may be suitable for healthy adults at high risk of **dengue** fever, additional clinical trials of this strain are warranted.

L16 ANSWER 15 OF 41 MEDLINE on STN
94219706. PubMed ID: 8166355. Immunization of monkeys with baculovirus-**dengue** type-4 recombinants containing envelope and nonstructural proteins: evidence of priming and partial protection. **Eckels K H**; Dubois D R; Summers P L; Schlesinger J J; Shelly M; Cohen S; Zhang Y M; Lai C J; Kurane I; Rothman A; +. (Walter Reed Army Institute of Research, Washington, District of Columbia.) The American journal of tropical medicine and hygiene, (1994 Apr) Vol. 50, No. 4, pp. 472-8. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

(**DEN**-4) recombinant-infected cell extracts. One recombinant contained all of the **DEN**-4 structural proteins and two nonstructural (NS) proteins (C-M-E-NS1-NS2a), while the other was a fusion protein containing a portion of the respiratory syncytial virus G glycoprotein and **DEN**-4 envelope glycoprotein (RSVG-E). Both preparations were immunogenic; all monkeys receiving either immunogen responded with the production of antivirion antibodies in enzyme immunoassays. All except one monkey receiving the recombinant b(C-M-E-NS1-NS2a) made antibodies to NS1. One monkey that received b(RSVG-E) showed the production of low levels of neutralizing antibodies. Following challenge with unmodified **DEN**-4 virus, seven of nine monkeys in the immunized group became infected and were viremic for a mean of 4.1 days. The control, sham-inoculated monkeys were also viremic; the mean number of days of viremia in this group was 4.7 days. The remaining monkeys in the immunized group (n = 7), although not protected, had evidence of priming. Hemagglutination inhibition antibody responses following challenge indicated an anamnestic response in this group of animals. Based on these results, it was concluded that future immunization schedules should be altered to optimize immune responses and that immunization with more potent and purified immunogens would probably result in higher seroconversion rates and antibody levels in monkeys.

L16 ANSWER 16 OF 41 MEDLINE on STN
92288605. PubMed ID: 1318137. New approaches to **flavivirus vaccine** development. Schlesinger J J; Putnak J R; **Eckels K H**. Biotechnology (Reading, Mass.), (1992) Vol. 20, pp. 289-307. Ref: 65. Journal code: 8300602. ISSN: 0740-7378. Pub. country: United States. Language: English.

L16 ANSWER 17 OF 41 MEDLINE on STN
91345181. PubMed ID: 1877715. Infection of Aedes albopictus and Aedes aegypti mosquitoes with **dengue** parent and progeny candidate **vaccine** viruses: a possible marker of human attenuation. Schoepp R J; Beaty B J; **Eckels K H**. (Department of Microbiology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins.) The American journal of tropical medicine and hygiene, (1991 Aug) Vol. 45, No. 2, pp. 202-10. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB **Dengue** (**DEN**-1) and **DEN**-4 parent (P) and progeny candidate **vaccine** (CV) viruses were compared in their abilities to infect and to replicate in Aedes aegypti and Aedes albopictus mosquitoes. The **DEN** CV clones were temperature sensitive (ts) and had small plaque morphology. The **DEN**-1 and **DEN**-4 CV viruses differed in their ability to infect, to replicate in, and to be transmitted by mosquitoes. The **DEN**-1 CV virus was not attenuated for the vector mosquitoes; oral infection rates with the CV virus were as high as or higher than the P virus, and the CV virus replicated efficiently in mosquitoes after oral infection. The **DEN**-4 CV virus was attenuated; it was less efficient than its P virus in infection and replication in mosquitoes. Thus, the ts phenotype and small plaque morphology are not reliable biological markers for prediction of vector attenuation. Similar results were reported by others for attenuation in man and monkeys. These studies with **DEN**-1 and **DEN**-4 viruses, and previously reported studies with **DEN**-2 virus and with **DEN**-3 virus suggest that vector and vertebrate host attenuation are genetically linked. Thus, vector attenuation may be a biological marker for human attenuation.

L16 ANSWER 18 OF 41 MEDLINE on STN
91341013. PubMed ID: 1651954. Large-scale purification of inactivated hepatitis A virus by centrifugation in non-ionic gradients. Dubois D R; **Eckels K H**; Ticehurst J; Binn L N; Timchak R L; Barvir D A; Rankin C T; O'Neill S P. (Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.) Journal of virological methods, (1991 May) Vol. 32, No. 2-3, pp. 327-34. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB Formalin-inactivated hepatitis A virus (HAV) can be purified for **vaccine** preparation by centrifugation in Renografin-76 (diatrizoate meglumine and diatrizoate sodium) gradients. Both continuous-flow rate-zonal and isopycnic methods were used for the separation of a major antigen component from minor antigen and host protein. The major antigen component, which appeared to contain complete virions by electron microscopy, could be recovered from gradients and accounted for approximately one third of the total antigen in the starting material. The HAV-specific purified antigen could be enriched 200-300-fold by either centrifugation procedure. The purified HAV antigen, when adsorbed to alum and inoculated into mice, was found to be highly immunogenic.

L16 ANSWER 19 OF 41 MEDLINE on STN
91326965. PubMed ID: 2101475. Expression of the envelope antigen of **dengue** virus in **vaccine** strains of Salmonella. Cohen S; Powell C J; Dubois D R; Hartman A; Summers P L; **Eckels K H**. (Biologics Research Dept., Walter Reed Army Institute of Research, Washington, D.C. 20307.)

Journal code: 8907468. ISSN: 0923-2508. Pub. country: France. Language: English.

AB The envelope gene of **dengue** 4 virus (**DEN**) was cloned in a plasmid under the control of Escherichia coli expression signals. A clone that expressed 93% of the gene was found to be detrimental to the bacterial host. Another clone which carried only 76% of the E gene was found to be quite stable in vitro as well as in vivo. The killed recombinant bacteria induced antibodies in mice which recognized native **DEN** virus. Attenuated Salmonella typhimurium (SAL) strains carrying the **DEN**-E plasmid were tested for their efficacy as orally administered live **vaccines**. Protective immunization was assessed in a mouse model by immunizing three-week old BALB/c mice followed by challenge with **DEN** virus. It was found that these young mice were highly susceptible to the carrier SAL strains (M206 and aroA SL3261). Moreover, the SAL-infected mice were more susceptible to **DEN** virus challenge than control mice, suggesting that the SAL infection caused immunosuppression in these young mice.

L16 ANSWER 20 OF 41 MEDLINE on STN

90358303. PubMed ID: 2389825. Preparation of an attenuated **dengue** 4 (341750 Carib) virus **vaccine**. II. Safety and immunogenicity in humans. Hoke C H Jr; Malinoski F J; **Eckels K H**; Scott R M; Dubois D R; Summers P L; Simms T; Burrous J; Hasty S E; Bancroft W H. (Walter Reed Army Institute of Research, Washington, DC.) The American journal of tropical medicine and hygiene, (1990 Aug) Vol. 43, No. 2, pp. 219-26. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB To determine safety and immunogenicity, a single 0.5 ml dose of a monovalent live-attenuated **dengue** (**DEN**) 4 (341750 Carib) **vaccine** was given sc to 3 groups of **flavivirus** nonimmune volunteers in increasing concentrations. Two recipients received 10(3) plaque forming units (PFU)/dose (1:100 dilution of stock **vaccine**). One remained asymptomatic, but became viremic between days 12 and 15, experienced a mild elevation of temperature (37.4 degrees C), and developed **DEN**-4 specific antibody. Neither recipient of the 10(4) PFU became infected. Eight volunteers then received undiluted **vaccine** (10(5) PFU). Viremia and antibody (neutralizing, hemagglutination inhibition, and IgM) developed in 5 of the 8 (63%). These 5 volunteers also developed a scarcely noticeable macular, blanching rash and minimal temperature elevations (37.3, 38.1, 37, 37.9, and 37.9 degrees C). Clinically insignificant decreases in total white blood cell, lymphocyte, and polymorphonuclear cell counts and an elevation in mononuclear cell counts occurred in association with viremia. This **vaccine** is safe, reasonably immunogenic, and suitable for further evaluation.

L16 ANSWER 21 OF 41 MEDLINE on STN

90358302. PubMed ID: 1975159. Preparation of an attenuated **dengue** 4 (341750 Carib) virus **vaccine**. I. Pre-clinical studies. Marchette N J; Dubois D R; Larsen L K; Summers P L; Kraiselburd E G; Gubler D J; **Eckels K H**. (University of Hawaii, Honolulu.) The American journal of tropical medicine and hygiene, (1990 Aug) Vol. 43, No. 2, pp. 212-8. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB **Dengue** 4 (**DEN**-4) virus strain 341750 Carib was modified by serial passage in primary canine kidney (PCK) cell cultures. By the 15th PCK passage, this virus was less infectious for monkeys and resulted in a significantly reduced viremia as compared to the parent **DEN**-4 virus. The 30th PCK passage of **DEN**-4 341750 Carib was non-infectious for monkeys. A **vaccine** prepared at the 20th PCK passage in DBS-FRhL-2 cells stimulated the production of both neutralizing and hemagglutination inhibition antibodies in monkeys; these animals were also protected against challenge with the homologous strain as well as a heterologous strain of **DEN**-4. An ID50 titration in monkeys resulted in a titer of greater than 10(4) plaque-forming units (PFU) for the **vaccine** virus and 0.5 PFU for the parent virus. Reduced monkey infectivity of this magnitude has been correlated with human attenuation in previous **dengue** **vaccine** candidates. The **DEN**-4 strain 341750 Carib PCK-20/FRhL-4 **vaccine** has been characterized and sufficiently tested to be considered for safety and immunogenicity trials in humans.

L16 ANSWER 22 OF 41 MEDLINE on STN

90145351. PubMed ID: 2301711. **Dengue** 3 virus infection of Aedes albopictus and Aedes aegypti: comparison of parent and progeny candidate **vaccine** viruses. Schoepp R J; Beaty B J; **Eckels K H**. (College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins.) The American journal of tropical medicine and hygiene, (1990 Jan) Vol. 42, No. 1, pp. 89-96. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB **DEN**-3 parent (strain CH53489) and progeny candidate **vaccine** (clone 24/28) viruses were compared in their abilities to interact with Aedes aegypti and Ae. albopictus. The parent and progeny virus were equivalent in their ability to infect, to replicate in, and to be transmitted by both species of mosquitoes. The candidate **vaccine** **DEN**-3 clone was

markers remained stable during mosquito passage. Thus, temperature sensitivity and small plaque morphology are not reliable biological markers for attenuation.

L16 ANSWER 23 OF 41 MEDLINE on STN
89259070. PubMed ID: 2724416. Mice immunized with recombinant **vaccinia** virus expressing **dengue** 4 virus structural proteins with or without nonstructural protein NS1 are protected against fatal **dengue** virus encephalitis. Bray M; Zhao B T; Markoff L; **Eckels K H**; Chanock R M; Lai C J. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.) Journal of virology, (1989 Jun) Vol. 63, No. 6, pp. 2853-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB We have constructed **vaccinia** virus recombinants expressing **dengue** virus proteins from cloned DNA for use in experimental immunoprophylaxis. A recombinant virus containing a 4.0-kilobase DNA sequence that codes for three structural proteins, capsid (C), premembrane (pre-M), and envelope (E), and for nonstructural proteins NS1 and NS2a produced authentic pre-M, E, and NS1 in infected CV-1 cells. Mice immunized with this recombinant were protected against an intracerebral injection of 100 50% lethal doses of **dengue** 4 virus. A recombinant containing only genes C, pre-M, and E also induced solid resistance to challenge. Deletion of the putative C-terminal hydrophobic anchor of the E glycoprotein did not result in secretion of E from recombinant-virus-infected cells. Recombinants expressing only the E protein preceded by its own predicted N-terminal hydrophobic signal or by the signal of influenza A virus hemagglutinin or by the N-terminal 71 amino acids of the G glycoprotein of respiratory syncytial virus produced glycosylated E protein products of expected molecular sizes. These **vaccinia** virus recombinants also protected mice.

L16 ANSWER 24 OF 41 MEDLINE on STN
89009999. PubMed ID: 3171230. Virulence of a live **dengue** virus **vaccine** candidate: a possible new marker of **dengue** virus attenuation. Innis B L; **Eckels K H**; Kraiselburd E; Dubois D R; Meadors G F; Gubler D J; Burke D S; Bancroft W H. (Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, D.C.) The Journal of infectious diseases, (1988 Oct) Vol. 158, No. 4, pp. 876-80. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

L16 ANSWER 25 OF 41 MEDLINE on STN
88275076. PubMed ID: 2969058. Immunization of mice with **dengue** structural proteins and nonstructural protein NS1 expressed by baculovirus recombinant induces resistance to **dengue** virus encephalitis. Zhang Y M; Hayes E P; McCarty T C; Dubois D R; Summers P L; **Eckels K H**; Chanock R M; Lai C J. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.) Journal of virology, (1988 Aug) Vol. 62, No. 8, pp. 3027-31. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB We have constructed a recombinant baculovirus containing a 4.0-kilobase **dengue** virus cDNA sequence that codes for the three virus structural proteins, capsid (C) protein, premembrane (PreM) protein, and envelope glycoprotein (E), and nonstructural proteins NS1 and NS2a. Infection of cultured Spodoptera frugiperda cells with this recombinant virus resulted in the production of E and NS1 proteins that were similar in size to the corresponding viral proteins expressed in **dengue** virus-infected simian cells. Other **dengue** virus-encoded proteins such as PreM and C were also synthesized. Rabbits immunized with the **dengue** virus protein products of the recombinant virus developed antibodies to PreM, E, and NS1, although the titers were low, especially to PreM and E. Nevertheless, the **dengue** virus antigens produced by the recombinant virus induced resistance in mice to fatal **dengue** encephalitis.

L16 ANSWER 26 OF 41 MEDLINE on STN
88062958. PubMed ID: 3316711. Expression of **dengue** virus structural proteins and nonstructural protein NS1 by a recombinant **vaccinia** virus. Zhao B T; Prince G; Horswood R; **Eckels K**; Summers P; Chanock R; Lai C J. (Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.) Journal of virology, (1987 Dec) Vol. 61, No. 12, pp. 4019-22. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB A recombinant **vaccinia** virus containing cloned DNA sequences coding for the three structural proteins and nonstructural proteins NS1 and NS2a of **dengue** type 4 virus was constructed. Infection of CV-1 cells with this recombinant virus produced **dengue** virus structural proteins as well as the nonstructural protein NS1. These proteins were precipitated by specific antisera and exhibited the same molecular size and glycosylation patterns as authentic **dengue** virus proteins. Infection of cotton rats with the recombinant virus induced NS1 antibodies in 1 of 11 animals. However, an immune response to the PreM and E glycoproteins was not detected. A reduced level of gene expression was probably the reason for the limited serologic response to these **dengue** virus antigens.

L16 ANSWER 27 OF 41 MEDLINE on STN
87154130. PubMed ID: 3826504. Lack of attenuation of a candidate **dengue** 1 **vaccine** (45AZ5) in human volunteers. McKee K T Jr; Bancroft W H; **Eckels K H**; Redfield R R; Summers P L; Russell P K. The American journal of tropical medicine and hygiene, (1987 Mar) Vol. 36, No. 2, pp. 435-42. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB A **dengue** type 1, candidate live virus **vaccine** (45AZ5) was prepared by serial virus passage in fetal rhesus lung cells. Infected cells were treated with a mutagen, 5-azacytidine, to increase the likelihood of producing attenuated variants. The **vaccine** strain was selected by cloning virus that produced only small plaques in vitro and showed reduced replication at high temperatures (temperature sensitivity). Although other candidate live **dengue** virus **vaccines** selected for similar growth characteristics have been attenuated for humans, two recipients of the 45AZ5 virus developed unmodified acute **dengue** fever. Viremia was observed within 24 hr of inoculation and lasted 12 to 19 days. Virus isolates from the blood produced large plaques in cell culture and showed diminished temperature sensitivity. The 45AZ5 virus is unacceptable as a **vaccine** candidate. This experience points out the uncertain relationship between in vitro viral growth characteristics and virulence factors for humans.

L16 ANSWER 28 OF 41 MEDLINE on STN
86060912. PubMed ID: 4067313. The association of enhancing antibodies with seroconversion in humans receiving a **dengue**-2 live-virus **vaccine**. **Eckels K H**; Kliks S C; Dubois D R; Wahl L M; Bancroft W H. Journal of immunology (Baltimore, Md. : 1950), (1985 Dec) Vol. 135, No. 6, pp. 4201-3. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB A group of human subjects, some with yellow fever (YF) antibodies, volunteered for testing of a live-attenuated **dengue**-2 (**DEN**-2) **vaccine**. Serum samples taken before **DEN**-2 **vaccination** were tested for their ability to enhance infection of human monocytes by **DEN**-2 virus. A significantly greater proportion of enhancing antibodies (Eab) were found in YF-immune (YFI) individuals (50%) as compared to those with no evidence of **flavivirus** infection (9.5%). Geometric mean titers of neutralizing and hemagglutination inhibiting antibodies to **DEN**-2 virus in YFI subjects with Eab were fourfold to seven-fold higher than in the YFI subjects without Eab in prevaccine sera and 10- to 35-fold higher than in non-immune volunteers. Additionally, levels of Eab in prevaccine sera were directly related to antibody titers found in postvaccine sera. The presence of Eab in the serum of a human subject before **DEN**-2 **vaccination** was a good predictor of the immune response after **vaccination**, and may in part be responsible for the higher seroconversion rate in YF immunes (90%) as compared to nonimmunes (61%) receiving this **vaccine**. This is the first human study to demonstrate that circulating Eab in non-**DEN**-immune persons is associated with an augmented immune response to **DEN** virus infection. This finding supports the hypothesis that cross-reactive antibodies against one **flavivirus** enhance an infection with another closely related **flavivirus**.

L16 ANSWER 29 OF 41 MEDLINE on STN
84304707. PubMed ID: 6476216. Selection of attenuated **dengue** 4 viruses by serial passage in primary kidney cells. V. Human response to immunization with a candidate **vaccine** prepared in fetal rhesus lung cells. **Eckels K H**; Scott R M; Bancroft W H; Brown J; Dubois D R; Summers P L; Russell P K; Halstead S B. The American journal of tropical medicine and hygiene, (1984 Jul) Vol. 33, No. 4, pp. 684-9. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB A **dengue** 4 (strain H241, PDK35-TD3 FRhL p3) **vaccine** attenuated by passage in primary dog kidney cells followed by passage and final **vaccine** preparation in DBS-FRhL-2 cells was tested in five yellow fever-immune volunteers. Only two volunteers seroconverted by producing hemagglutination-inhibiting and neutralizing antibodies. Mild illness, compatible with **dengue** infection was found only in the individuals who later developed antibodies. Both volunteers developed a rash by the 8th day following **vaccination**, coinciding with a slight elevation in temperature and leukopenia. Additionally, several serum enzymes were elevated during the observation period. **Dengue** 4 virus was isolated from the blood of the two infected volunteers starting as early as day 5 post **vaccination**. During the viremic period, which lasted 5 days, phenotypically-changed virus was recovered, indicating genetic instability of the **vaccine** virus. The clinical disease and immune response in the two infected individuals was probably related to replication of the variant virus. Further testing of this **vaccine** in its present form is not indicated.

L16 ANSWER 30 OF 41 MEDLINE on STN
84304706. PubMed ID: 6476215. Selection of attenuated **dengue** 4 viruses by serial passage in primary kidney cells. IV. Characterization of a **vaccine** candidate in fetal rhesus lung cells. Halstead S B; **Eckels K**

tropical medicine and hygiene, (1984 Jul) Vol. 33, No. 4, pp. 679-83. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB A strain of primary dog kidney (PDK)-passaged **dengue** (**DEN**) 4 (H-241) virus cloned by terminal dilution (PDK 35-TD3) was propagated in large volumes in fetal rhesus lung (FRhL) cells to produce a candidate **vaccine** for evaluation in man. Production seed (FRhL p2) and candidate **vaccine** (FRhL p3) were subjected to rigorous safety tests to exclude contaminating microbial agents. There was no significant monkey neurovirulence of parental or PDK-passaged **DEN**-4 virus or of control fluid cultures. FRhL-passaged viruses retained the phenotypic characteristics: small (occasional medium) plaque; temperature sensitivity at 38.5 degrees C; and absence of plaque formation in African green monkey kidney cells, cytopathic effect in LLC-MK2 cells, and viral growth in human monocytes. FRhL p2 virus displayed low virulence for monkeys; only one of four animals was viremic and three of four developed low-titered antibody. FRhL p3 virus produced viremia in three monkeys and moderate to high hemagglutination-inhibition and neutralizing antibody titers in all animals. Virus at both passages in FRhL exhibited reduced neurovirulence in suckling mice as compared to parental **DEN**-4. Because of its safety and desirable monkey virulence attributes PDK 35-TD3 FRhL p3 is recommended for human phase I trial.

L16 ANSWER 31 OF 41 MEDLINE on STN

84241242. PubMed ID: 6376649. **Dengue** virus type 2 **vaccine**: reactogenicity and immunogenicity in soldiers. Bancroft W H; Scott R M; **Eckels K H**; Hoke C H Jr; Simms T E; Jesrani K D; Summers P L; Dubois D R; Tsoulos D; Russell P K. The Journal of infectious diseases, (1984 Jun) Vol. 149, No. 6, pp. 1005-10. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB A live **dengue** virus type 2 (**dengue**-2) **vaccine** (PR-159/S-1) was tested for reactogenicity and immunogenicity in a placebo-controlled, double-blind clinical trial involving 98 soldiers. Seroconversion rates based on the development of neutralizing antibody to **dengue**-2 were 90% in 70 recipients with immunity to yellow fever and 61% in 28 **vaccinees** without such immunity (P less than .01). Peak titers of neutralizing antibody were three times higher in recipients with antibody to yellow fever virus and persisted in most for at least 18 months. Individuals seroconverting to the **vaccine** virus more frequently experienced systemic symptoms than those who received placebo (P less than .02). Future users of this **dengue**-2 **vaccine** may wish to employ immunization schedules that include preliminary immunization against yellow fever and must be prepared to accept mild **vaccine**-related symptoms in some recipients.

L16 ANSWER 32 OF 41 MEDLINE on STN

84240227. PubMed ID: 6330164. Antibody response to **dengue**-2 **vaccine** measured by two different radioimmunoassay methods. Summers P L; **Eckels K H**; Dalrymple J M; Scott R M; Boyd V A. Journal of clinical microbiology, (1984 May) Vol. 19, No. 5, pp. 651-9. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB Two different radioimmunoassays were used to detect virus-specific antibodies in sera from human volunteers inoculated with an attenuated **dengue** type 2 (**DEN**-2) **vaccine** (PR-159/S-1). An indirect radioimmunoassay required purified **DEN**-2 virions for optimal reactivity but was 10 to 500 times more sensitive than neutralization or hemagglutination inhibition tests. An antibody capture radioimmunoassay was able to utilize crude antigens from either **DEN**-infected mouse brains or Aedes albopictus cell culture supernatants. When the two radioimmunoassay techniques were compared, the indirect method appeared to be the best assay for immunoglobulin G (IgG), whereas the antibody capture method was more sensitive for IgM detection. Selected human sera were examined for IgG, IgM, and IgA responses by using both techniques at various intervals after immunization. Although there were differences in magnitude, yellow fever immune as well as **flavivirus** nonimmune volunteers responded to **DEN**-2 **vaccination** by demonstrating IgG, IgM, and IgA antibody responses. In the nonimmune group, the most prevalent immunoglobulin detected was IgM, whereas in the yellow fever immune group, the predominant post-**DEN**-2 **vaccine** immunoglobulin was IgG. The preponderance of **DEN**-2-specific neutralizing antibodies were associated with either IgM or IgG according to the immune status of the volunteer. All classes of immunoglobulins attained maximum levels between 21 and 60 days postvaccination. In the majority of volunteers, IgM responses were relatively transient and could not be detected 6 months after immunization, whereas IgG and IgA antibodies were still detectable after this period.

L16 ANSWER 33 OF 41 MEDLINE on STN

84089195. PubMed ID: 6655288. **Dengue** 2 **vaccine**: dose response in volunteers in relation to yellow fever immune status. Scott R M; **Eckels K H**; Bancroft W H; Summers P L; McCown J M; Anderson J H; Russell P K. The Journal of infectious diseases, (1983 Dec) Vol. 148, No. 6, pp. 1055-60. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States.

AB A live **dengue** 2 **vaccine** was tested in 38 volunteers in an evaluation of the safety, infectivity, and immunogenicity of doses of 10(1.8)-10(5.5) plaque-forming units. Twenty yellow fever-immune and 18 yellow fever-nonimmune individuals received 0.5 ml of **vaccine** sc. Immunization was dose related in yellow fever-immune volunteers, with a 50% immunizing dose of 10(3.3) plaque-forming units. In the group not immune to yellow fever, some but not all recipients of each **vaccine** dilution were immunized, and no 50% immunizing dose could be estimated. Volunteers immune to yellow fever developed adequate titers of neutralizing antibody to **dengue** 2 virus and maintained them for at least three years; those not immune to yellow fever developed lower antibody titers that disappeared within six months in half of the cases. More than 40 isolates of **dengue** 2 virus from 12 volunteers retained the in vitro growth characteristics of the **vaccine** virus; this result affirmed the genetic stability of the virus. Common clinical signs in immunized individuals were leukopenia (55%), macular rash (15%), and fever (10%).

L16 ANSWER 34 OF 41 MEDLINE on STN
83072305. PubMed ID: 7149108. **Dengue**-2 **vaccine**: oral infection, transmission, and lack of evidence for reversion in the mosquito, Aedes aegypti. Miller B R; Beaty B J; Aitken T H; **Eckels K H**; Russell P K. The American journal of tropical medicine and hygiene, (1982 Nov) Vol. 31, No. 6, pp. 1232-7. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB The **dengue**-2 **vaccine** virus (S-1), and its parent virus (PR-159), were compared for their ability to infect orally, to replicate in, and subsequently to be transmitted by Aedes aegypti mosquitoes. The **vaccine** virus was markedly less efficient in its ability to infect mosquitoes orally. After ingesting infectious bloodmeals containing 3, 7 to 8.2 log10MID50/ml of the respective viruses, 56% (220/396) of the mosquitoes became orally infected with the parent virus contrasted with 16% (66/397) for the **vaccine** virus. None of the 16 infected mosquitoes transmitted the **vaccine** virus, while 14% (3/22) of the mosquitoes transmitted the parent virus. The **vaccine** virus remained temperature-sensitive (restrictive temperature 39 degrees C) after orally infecting and replicating in Ae. aegypti mosquitoes.

L16 ANSWER 35 OF 41 MEDLINE on STN
83072304. PubMed ID: 7149107. **Dengue**-2 **vaccine**: infection of Aedes aegypti mosquitoes by feeding on viremic recipients. Bancroft W H; Scott R M; Brandt W E; McCown J M; **Eckels K H**; Hayes D E; Gould D J; Russell P K. The American journal of tropical medicine and hygiene, (1982 Nov) Vol. 31, No. 6, pp. 1229-31. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB Colonized Aedes aegypti mosquitoes were fed on voluntary recipients of an experimental, live, attenuated, **dengue** type 2 (PR 159/S-1) **vaccine** to estimate the frequency of vector infection and the stability of the virus in mosquitoes. Two volunteers were viremic at the time of mosquito feeding, but only two of 114 mosquitoes that took a viremic blood meal became infected with the **vaccine** virus. Strains of virus recovered from the bodies of the mosquitoes and the volunteer's blood retained the temperature sensitivity and small plaque growth characteristics of the **vaccine** virus. **Dengue** viral antigen was not detectable in any of the mosquito heads by direct immunofluorescence and in vitro virus transmission by droplet feeding was not observed. This experiment showed that vector mosquitoes can be infected with **vaccine** virus by feeding on viremic **vaccinees**. Furthermore, the virus is sufficiently stable to retain the in vitro growth characteristics associated with the **vaccine** virus.

L16 ANSWER 36 OF 41 MEDLINE on STN
81166976. PubMed ID: 7216469. **Dengue**-2 **vaccine**: virological, immunological, and clinical responses of six yellow fever-immune recipients. Bancroft W H; Top F H Jr; **Eckels K H**; Anderson J H Jr; McCown J M; Russell P K. Infection and immunity, (1981 Feb) Vol. 31, No. 2, pp. 698-703. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB Six male volunteers, previously immunized with yellow fever **vaccine**, were inoculated subcutaneously with a live, attenuated **dengue**-2 virus (PR-159/S-1) candidate **vaccine**. Five recipients developed viremia 8 or 9 days after **vaccination**, which lasted 1 to 10 days. The onset of viremia was followed by fever in three people, transient leukopenia in four, and an erythematous rash in one. One volunteer developed an oral temperature of 38.8 degrees C with headache, myalgia, fatigue, and photophobia suggestive of mild **dengue** fever. All five viremic volunteers developed fourfold or greater rises in serum neutralizing antibody. The sixth volunteer, who had a low titer of preexisting **dengue**-2 neutralizing antibody, had no viremia, no symptoms, and a modest rise in hemagglutination inhibiting antibody. Virus isolates obtained from plasma retained the small-plaque and temperature-sensitive growth characteristics of the **vaccine** virus in vitro. In this study, the **vaccine** virus genetically stable and immunogenic and seemed

L16 ANSWER 37 OF 41 MEDLINE on STN
80136317. PubMed ID: 6766903. **Dengue**-2 **vaccine**: viremia and immune responses in rhesus monkeys. Scott R M; Nisalak A; **Eckels K H**; Tingpalapong M; Harrison V R; Gould D J; Chapple F E; Russell P K. Infection and immunity, (1980 Jan) Vol. 27, No. 1, pp. 181-6. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

L16 ANSWER 38 OF 41 MEDLINE on STN
80136316. PubMed ID: 6766902. **Dengue**-2 **vaccine**: preparation from a small-plaque virus clone. **Eckels K H**; Harrison V R; Summers P L; Russell P K. Infection and immunity, (1980 Jan) Vol. 27, No. 1, pp. 175-80. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB The S-1 clone of **dengue** type 2 virus was used for the preparation of a live-attenuated **vaccine** after passage in DBS-FRhL-2 cell culture. The **vaccine** virus had a relatively higher replicative capacity at superoptimal temperatures than its precursor virus, S-1, passaged in primary green monkey kidney cells (S-1 PGMK). There was also a tendency for the S-1 **vaccine** virus to exhibit leakiness at increased temperatures. Another in vitro marker, replication in monkey peripheral blood leukocytes, indicated less host restriction for the S-1 **vaccine** in comparative assays with S-1 PGMK virus. Mouse virulence appeared to remain stable on passage in DBS-FRhL-2 cells, whereas monkey immunogenicity decreased. Cautious trials of the **dengue** type 2 S-1 **vaccine** in humans are indicated.

L16 ANSWER 39 OF 41 MEDLINE on STN
78004976. PubMed ID: 409682. Virulence and immunogenicity of a temperature-sensitive **dengue**-2 virus in lower primates. Harrison V R; **Eckels K H**; Sagartz J W; Russell P K. Infection and immunity, (1977 Oct) Vol. 18, No. 1, pp. 151-6. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB Clones of **dengue**-2 virus were tested for virulence by inoculation of rhesus monkeys and chimpanzees. Although primates showed no overt signs of illness, inoculation with the parent virus or a subline of a large-plaque clone resulted in a viremia lasting 1 to 7 days. By these criteria, sublines of a small-plaque clone were significantly less virulent and produced little or no viremia in primate hosts. Although they had a substantially reduced viremia, primates inoculated with the small-plaque sublines showed stimulation of complement-fixing, hemagglutination-inhibiting, and neutralizing antibodies. The protection afforded rhesus monkeys 3 months after inoculation with two of the small-plaque sublines was demonstrated by a lack of viremia and a failure to escalate preexisting antibody levels after challenge with the parent virus. Both the S-1 subline and the parent virus had a limited capacity to produce central nervous system pathology in monkeys inoculated intrathalamically and intrathecally. Evidence thus far accumulated for primates indicates that the S-1 subline of **dengue**-2 virus has potential value as a candidate **vaccine** virus.

L16 ANSWER 40 OF 41 MEDLINE on STN
77027805. PubMed ID: 977127. Isolation of a temperature-sensitive **dengue**-2 virus under conditions suitable for **vaccine** development. **Eckels K H**; Brandt W E; Harrison V R; McCown J M; Russell P K. Infection and immunity, (1976 Nov) Vol. 14, No. 5, pp. 1221-7. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB **Dengue** virus, type 2, in viremic human sera and after passage in cell cultures produces mixtures of small and large plaques when assayed in LLC-MK2 cells. Clones of **dengue** virus type 2 obtained by plaque selection in primary green monkey kidney cell cultures were tested for temperature sensitivity in vitro and for virulence by intracerebral inoculation of suckling mice. Sublines of a small-plaque clone were found to have lower nonpermissive temperatures than the parent virus by both plaque formation and release of infectious virus into the culture media. Small-plaque sublines were significantly less virulent in suckling mice than was the parent virus. Sublines from a large-plaque clone were not temperature sensitive and closely resembled parent virus mixed-plaque morphology. When small-plaque sublines were serially passaged using undiluted inocula, reversion occurred as evidenced by the appearance of large plaques and return of mouse virulence. Small-plaque virus could be maintained through several serial passages without reversion by using low-input inocula. Desirable passage history as well as temperature-sensitive and attentuation characteristics of the S-1 small-plaque subline make it appear suitable as a **vaccine** candidate virus.

L16 ANSWER 41 OF 41 MEDLINE on STN
70155395. PubMed ID: 4985431. Chikungunya virus **vaccine** prepared by Tween-ether extraction. **Eckels K H**; Harrison V R; Hetrick F M. Applied microbiology, (1970 Feb) Vol. 19, No. 2, pp. 321-5. Journal code: 7605802. ISSN: 0003-6919. Pub. country: United States. Language: English.

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E1            2     PUTMANS PASCALE/AU
E2            1     PUTNA L/AU
E3           19 --> PUTNAK J R/AU
E4            2     PUTNAK J ROBERT/AU
E5           10     PUTNAK R/AU
E6            5     PUTNAK ROBERT/AU
E7            1     PUTNAK ROBERT J/AU
E8            5     PUTNAM A/AU
E9            1     PUTNAM A D/AU
E10           6     PUTNAM A H/AU
E11           4     PUTNAM A J/AU
E12           2     PUTNAM A L/AU

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L17          21 ("PUTNAK J R"/AU OR "PUTNAK J ROBERT"/AU)

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     FILE 'USPATFULL' ENTERED AT 11:02:14 ON 27 JUN 2006
                  E ECKELS KENNETH/IN
L1            11 S E3-E4
                  E PUTNAK JOSEPH R/IN
L2             9 S E2-E3
L3             0 S L1 NOT L1
                  E DUBOIS DORIA R/IN
L4             6 S E3
L5             0 S L4 NOT L1

     FILE 'WPIDS' ENTERED AT 11:03:30 ON 27 JUN 2006
                  E ECKELS K/IN
L6            13 S E3 OR E4
                  E PUTNAK J R/IN
L7            12 S E3
L8             3 S L7 NOT L6
                  E DUBOIS D R/IN
L9            29 S E3
L10           20 S L9 NOT (L6 OR L8)
L11            1 S L10 AND (DEN? OR FLAVIVIR?)

     FILE 'MEDLINE' ENTERED AT 11:05:22 ON 27 JUN 2006
                  E ECKELS K/AU
L12           64 S E3-E5
L13           50 S L12 AND (FLAVIVIR? OR DEN?)
L14           42 S L13 AND VACCIN?
L15            1 S L14 AND (MULTIVALENT)
L16           41 S L14 NOT L15
                  E PUTNAK J R/AU
L17           21 S E3-E4

=> s l17 not l16
L18           17 L17 NOT L16

=> s l18 and (flavivir? or deng?)
           3739 FLAVIVIR?
           5288 DENG?
L19           11 L18 AND (FLAVIVIR? OR DENG?)

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L19  ANSWER 1 OF 11     MEDLINE on STN
2004040704.     PubMed ID: 14740953.   Atypical antibody responses in dengue
     vaccine recipients. Kanesa-Thasan N; Sun W; Ludwig G V; Rossi C; Putnak J
     R; Mangiafico J A; Innis B L; Edelman R. (Walter Reed Army Institute of
     Research, Washington, District of Columbia, USA.. niranjan.kanesa-
     thasan@det.amedd.army.mil) . The American journal of tropical medicine and
     hygiene, (2003 Dec) Vol. 69, No. 6 Suppl, pp. 32-8.  Journal code:
     0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.
AB   Eight of 69 (12%) healthy adult volunteers vaccinated with monovalent
     live-attenuated dengue virus (DENV) vaccine candidates had atypical
     antibody responses, with depressed IgM:IgG antibody ratios and induction
     of high-titer hemagglutination-inhibiting and neutralizing (NT) antibodies
     to all four DENV serotypes.  These features suggested flavivirus
     exposure prior to DENV vaccination, yet no volunteer had a history of
     previous flavivirus infection, flavivirus vaccination, or antibody to
     flaviviruses evident before DENV vaccination.  Moreover, production of
     antibody to DENV by atypical responders (AR) was not accelerated compared
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Further evaluation revealed no differences in sex, age, race, DENV vaccine candidate received, or clinical signs and symptoms following vaccination between AR and NR. However, viremia was delayed at the onset in AR compared with NR. A comparative panel of all AR and five randomly selected NR found **flavivirus** cross-reactive antibody after vaccination only in AR. Unexpectedly, six of eight AR had NT antibodies to yellow fever virus (YFV) > 1:10 before vaccination while NR had none (P = 0.04). The AR also universally demonstrated YFV NT antibody titers > or = 1:160 after DENV vaccination, whereas four of five NR failed to seroconvert (P = 0.02). Yellow fever virus priming broadens the antibody response to monovalent DENV vaccination. The effect of **flavivirus** priming on the clinical and immunologic response to tetravalent DENV vaccine remains to be determined.

L19 ANSWER 2 OF 11 MEDLINE on STN

2002386869. PubMed ID: 12135278. Short report: absence of protective neutralizng antibodies to West Nile virus in subjects following vaccination with Japanese encephalitis or **dengue** vaccines. Kanesa-Thasan N; **Putnak J R**; Mangiafico J A; Saluzzo J E; Ludwig G V. (Walter Reed Army Institute of Research, Washington, District of Columbia 20307-5100, USA,.. niranjan.kanesa-thasan@det.amedd.army.mil) . The American journal of tropical medicine and hygiene, (2002 Feb) Vol. 66, No. 2, pp. 115-6. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB Protection of individuals against West Nile (WN) encephalitis is an emerging concern in the United States and Europe. We investigated whether immunization with licensed inactivated Japanese encephalitis (JE) vaccine or experimental live attenuated **dengue** vaccines resulted in induction of cross-neutralizing antibodies against WN virus. Protective neutralizing antibody titers to WN virus were not detected in any volunteer despite successful immunization to related **flaviviruses**. Vaccination against JE or **dengue** is unlikely to prevent WN virus infection but may still protect against disease.

L19 ANSWER 3 OF 11 MEDLINE on STN

2001237529. PubMed ID: 11304057. Limited potential for transmission of live **dengue** virus vaccine candidates by Aedes aegypti and Aedes albopictus. Sardelis M R; Edelman R; Klein T A; Innis B L; **Putnak J R**; Jones J W; Turell M J. (Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011, USA.) The American journal of tropical medicine and hygiene, (2000 Jun) Vol. 62, No. 6, pp. 698-701. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB To evaluate the transmission risk of four live **dengue** (DEN) vaccine candidates developed by the U.S. Army (DEN-1, 45AZ5 PDK 20; DEN-2, S16803 PDK 50; DEN-3, CH53489 PDK 20; and DEN-4, 341750 PDK 20), we tested 3,010 Aedes aegypti and 1,576 Aedes albopictus mosquitoes blood-fed on 21 volunteers who had been administered one of the four vaccine candidates or the licensed yellow fever (YF) vaccine (17D). We used an indirect immunofluorescence assay (IFA) to detect DEN or YF viral antigen in the heads of mosquitoes. Corresponding to the lack of a detectable viremia among volunteers inoculated 8-13 days previously with live DEN-1 or DEN-2 vaccine candidates, only six mosquitoes developed disseminated infections after feeding on these volunteers. These six mosquitoes included 4 of 247 Ae. albopictus fed on volunteers inoculated with the DEN-1 vaccine candidate and 2 of 528 Ae. aegypti fed on volunteers inoculated with the DEN-2 vaccine candidate. Infection was confirmed in each of these IFA-positive mosquitoes by isolating infectious virus from the mosquito's body in Vero-cell culture. None of the 1,252 or the 969 mosquitoes fed on DEN-3 or DEN-4 recipients, respectively, were infected. Overall, dissemination rates in Ae. albopictus and Ae. aegypti were low. Dissemination rates were 0.5%, 0.3%, < 0.1%, and < 0.1% for the DEN-1 through DEN-4 vaccine candidates, respectively. Because of the observed low dissemination rates, it is unlikely that these vaccine viruses would be transmitted under natural conditions.

L19 ANSWER 4 OF 11 MEDLINE on STN

2001222564. PubMed ID: 11312014. Safety and immunogenicity of attenuated **dengue** virus vaccines (Aventis Pasteur) in human volunteers. Kanesa-thasan N; Sun W; Kim-Ahn G; Van Albert S; **Putnak J R**; King A; Raengsakulsrach B; Christ-Schmidt H; Gilson K; Zahradnik J M; Vaughn D W; Innis B L; Saluzzo J F; Hoke C H Jr. (Walter Reed Army Institute of Research, Washington, DC, USA.. niranjan.kanesa-thasan@na.amedd.army.mil) . Vaccine, (2001 Apr 30) Vol. 19, No. 23-24, pp. 3179-88. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.

AB A randomized, controlled, double-blinded study was conducted to determine safety and immunogenicity of five live attenuated **dengue** vaccines produced by Aventis Pasteur (AvP). The study was completed with 40 **flavivirus** non-immune volunteers: five recipients of each monovalent (**dengue**-1, **dengue**-2, **dengue**-3, or **dengue**-4) vaccine, ten recipients of tetravalent (**dengue**-1, **dengue**-2, **dengue**-3, and

vaccines were administered in a single subcutaneous dose (range, 3.6-4.4 log(10) plaque forming units). No serious adverse reactions occurred in volunteers followed for 6 months after vaccination. Five vaccine recipients developed fever (T > or = 38.0 degrees C), including four tetravalent vaccinees between days 8 and 10 after vaccination. **Dengue**-1, **dengue**-2, **dengue**-3, or **dengue**-4 vaccine recipients reported similar frequency of mild symptoms of headache, malaise, and eye pain. Tetravalent vaccinees noted more moderate symptoms with onset from study days 8-11 and developed maculopapular rashes distributed over trunk and extremities. Transient neutropenia (white blood cells < 4000/mm3) was noted after vaccination but not thrombocytopenia (platelets < 100,000/mm3). All **dengue**-3, **dengue**-4, and tetravalent vaccine recipients were viremic between days 7 and 12 but viremia was rarely detected in **dengue**-1 or **dengue**-2 vaccinees. All **dengue**-2, **dengue**-3, and **dengue**-4, and 60% of **dengue**-1 vaccine recipients developed neutralizing and/or immunoglobulin M antibodies. All tetravalent vaccine recipients were viremic with **dengue**-3 virus and developed neutralizing antibodies to **dengue**-3 virus. Seven volunteers also had multivalent antibody responses, yet the highest antibody titers were against **dengue**-3 virus. The AvP live attenuated **dengue** virus vaccines are safe and tolerable in humans. The live attenuated tetravalent **dengue** vaccine was most reactogenic, and preferential replication of **dengue**-3 virus may have affected its infectivity and immunogenicity.

L19 ANSWER 5 OF 11 MEDLINE on STN
97398367. PubMed ID: 9256265. A putative cellular receptor for **dengue** viruses. **Putnak J R**; Kanesa-Thasan N; Innis B L. Nature medicine, (1997 Aug) Vol. 3, No. 8, pp. 828-9. Journal code: 9502015. ISSN: 1078-8956. Pub. country: United States. Language: English.

L19 ANSWER 6 OF 11 MEDLINE on STN
96118136. PubMed ID: 8578812. Mice immunized with a **dengue** type 2 virus E and NS1 fusion protein made in Escherichia coli are protected against lethal **dengue** virus infection. Srivastava A K; **Putnak J R**; Warren R L; Hoke C H Jr. (Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, DC 20307-5100, USA.) Vaccine, (1995 Sep) Vol. 13, No. 13, pp. 1251-8. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A gene fragment encoding the C-terminal 204 amino acids (AA) from the structural envelope glycoprotein (E) and the N-terminal 65 AA from non-structural protein one (NS1) of **dengue** type 2 virus (DEN-2) was expressed in Escherichia coli (E. coli) as a fusion protein with staphylococcal protein A. The recombinant fusion protein was purified and analysed for its antigenicity, its immunogenicity and its ability to protect mice against lethal challenge with live DEN-2 virus. The recombinant protein was found to be reactive with anti-DEN-2 polyclonal and monoclonal antibodies. Mice immunized with the purified fusion protein made anti-DEN-2 antibodies measured by the hemagglutination-inhibition (HI) and neutralization (N) tests, and were protected against lethal challenge with DEN-2 virus administered by intracranial inoculation.

L19 ANSWER 7 OF 11 MEDLINE on STN
91029155. PubMed ID: 2224837. The **dengue** viruses. Henchal E A; **Putnak J R**. (Department of Virus Diseases, Walter Reed Army Institute of Research, Washington 20307-5100.) Clinical microbiology reviews, (1990 Oct) Vol. 3, No. 4, pp. 376-96. Ref: 298. Journal code: 8807282. ISSN: 0893-8512. Pub. country: United States. Language: English.

AB **Dengue**, a major public health problem throughout subtropical and tropical regions, is an acute infectious disease characterized by biphasic fever, headache, pain in various parts of the body, prostration, rash, lymphadenopathy, and leukopenia. In more severe or complicated **dengue**, patients present with a severe febrile illness characterized by abnormalities of hemostasis and increased vascular permeability, which in some instances results in a hypovolemic shock. Four distinct serotypes of the **dengue** virus (**dengue**-1, **dengue**-2, **dengue**-3, and **dengue**-4) exist, with numerous virus strains found worldwide. Molecular cloning methods have led to a greater understanding of the structure of the RNA genome and definition of virus-specific structural and nonstructural proteins. Progress towards producing safe, effective **dengue** virus vaccines, a goal for over 45 years, has been made.

L19 ANSWER 8 OF 11 MEDLINE on STN
90362056. PubMed ID: 2144016. Protection of mice against yellow fever virus encephalitis by immunization with a vaccinia virus recombinant encoding the yellow fever virus non-structural proteins, NS1, NS2a and NS2b. **Putnak J R**; Schlesinger J J. (Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20307.) The Journal of general virology, (1990 Aug) Vol. 71 (Pt 8), pp. 1697-702. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

non-structural protein, NS1, has suggested its incorporation into possible recombinant vaccines. The region of the 17D yellow fever virus (YFV) genome encoding the C terminus of envelope glycoprotein and extending to the N terminus of non-structural protein NS3 (NS1-NS2a-NS2b; nucleotides 2030 to 4940) was expressed in vaccinia virus and physical and immunogenic properties of the NS1 moiety were studied. Recombinant NS1 protein, and native YFV NS1, was detected at the surface of infected cells by immunofluorescence and by immune cytolysis after treatment with anti-NS1 antibody and complement. NS1 was also detected in the extracellular medium as a secreted form. Recombinant NS1 was immunoprecipitated as a single protein of approximately the same size as native 17D YFV NS1. Unboiled, both recombinant and native NS1 formed polymers of high Mr. Immunization of mice with this recombinant vaccinia virus stimulated production of non-neutralizing, complement-fixing cytolytic antibody and conferred partial protection against lethal intracerebral inoculation of mice with live 17D YFV.

L19 ANSWER 9 OF 11 MEDLINE on STN
90188305. PubMed ID: 2138210. Cell surface expression of yellow fever virus non-structural glycoprotein NS1: consequences of interaction with antibody. Schlesinger J J; Brandriss M W; **Putnak J R**; Walsh E E. (Department of Medicine, Rochester General Hospital, New York.) The Journal of general virology, (1990 Mar) Vol. 71 (Pt 3), pp. 593-9. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Among antibodies to **flaviviral** proteins only those directed at the virion envelope protein (E) or the non-structural glycoprotein NS1 are known to confer protection. To investigate the possible role of complement-mediated cytolysis (CMC) in protection we measured the capacity of anti-NS1, or E monospecific serum or monoclonal antibodies to bind to yellow fever virus (YFV)-infected cells and of anti-NS1 or E serum to sensitize them to CMC. Although both anti-NS1 and anti-E antibody bound to YFV-infected cells, CMC was observed only with anti-NS1 antibody. Greater binding by anti-NS1 antibody suggested the presence of larger amounts of NS1 than E associated with the cell membrane. Using the cell membrane-impermeable, cross-linking reagent BS3, cell surface NS1, but not E, was detected as a homopolymer, a form in which bound antibody might be expected to activate complement more efficiently. Peak titres of progeny virus were reduced 10- to 100-fold when infected cells were treated with complement-fixing, anti-NS1 monoclonal antibody or monospecific, anti-NS1 rabbit serum and complement. Taken together these results are consistent with the hypothesis that CMC subserved by anti-NS1 antibody provides an alternative to direct neutralization of virus in the protective immune response to **flaviviral** infection.

L19 ANSWER 10 OF 11 MEDLINE on STN
90020399. PubMed ID: 2552624. Morphogenesis of **flaviviruses**. Hase T; Summers P L; Eckels K H; **Putnak J R**. Sub-cellular biochemistry, (1989) Vol. 15, pp. 275-305. Ref: 119. Journal code: 0316571. ISSN: 0306-0225. Pub. country: ENGLAND: United Kingdom. Language: English.

L19 ANSWER 11 OF 11 MEDLINE on STN
88160069. PubMed ID: 2964755. Functional and antigenic domains of the **dengue**-2 virus nonstructural glycoprotein NS-1. **Putnak J R**; Charles P C; Padmanabhan R; Irie K; Hoke C H; Burke D S. (Department of Virus Diseases, Walter Reed Army Institute of Research, Washington D.C. 20307.) Virology, (1988 Mar) Vol. 163, No. 1, pp. 93-103. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.
AB The gene coding for the nonstructural glycoprotein of **dengue**-2 virus was cloned, sequenced, and expressed in Escherichia coli. There was about 70% conservation at the amino acid level with **dengue** serotypes 1 and 4 suggesting an important common function for this protein. Conserved hydrophobic domains were found both before the amino-terminus and at the carboxy-terminus, consistent with transmembrane roles. Evidence for at least partial translocation of NS-1 through the inner membrane of E. coli was found. Also conserved were two signals for N-linked glycosylation located near the middle of NS-1. Various regions of NS-1 were tested for antigenicity with mouse and rabbit polyclonal and mouse monoclonal antibodies. The mouse polyclonal antibodies, made against a crude **dengue**-infected mouse brain immunogen, reacted most strongly with N-terminal regions of NS-1, whereas, the rabbit antiserum, made against purified NS-1 protein, reacted strongest with C-terminal regions. These findings suggest that immunogen presentation or species differences could be important. Although most of the monoclonals appeared to be unreactive in Western blots with expressed NS-1 proteins, two appeared to react strongly; the region from amino acid (a.a.) 273 to a.a. 346 was required for antibody binding. This region, located adjacent to the two conserved C-terminal hydrophobic domains, is highly charged and contains 5 of the 10 conserved cysteine residues of NS-1.

=> e dubois d r/au

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E2            1     DUBOIS D P/AU
E3           31 --> DUBOIS D R/AU
E4            2     DUBOIS D W/AU
E5           15     DUBOIS D Y/AU
E6          102     DUBOIS DALCQ M/AU
E7            9     DUBOIS DALCQ M E/AU
E8            8     DUBOIS DALCQ MONIQUE/AU
E9            1     DUBOIS DALE/AU
E10           1     DUBOIS DALQ M/AU
E11           3     DUBOIS DAMIEN/AU
E12           1     DUBOIS DAN/AU

=> s e3
L20          31 "DUBOIS D R"/AU

=> d his

     (FILE 'HOME' ENTERED AT 11:02:05 ON 27 JUN 2006)

     FILE 'USPATFULL' ENTERED AT 11:02:14 ON 27 JUN 2006
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                E PUTNAK JOSEPH R/IN
L2              9 S E2-E3
L3              0 S L1 NOT L1
                E DUBOIS DORIA R/IN
L4              6 S E3
L5              0 S L4 NOT L1

     FILE 'WPIDS' ENTERED AT 11:03:30 ON 27 JUN 2006
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                E PUTNAK J R/IN
L7             12 S E3
L8              3 S L7 NOT L6
                E DUBOIS D R/IN
L9             29 S E3
L10            20 S L9 NOT (L6 OR L8)
L11             1 S L10 AND (DEN? OR FLAVIVIR?)

     FILE 'MEDLINE' ENTERED AT 11:05:22 ON 27 JUN 2006
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L12            64 S E3-E5
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L14            42 S L13 AND VACCIN?
L15             1 S L14 AND (MULTIVALENT)
L16            41 S L14 NOT L15
                E PUTNAK J R/AU
L17            21 S E3-E4
L18            17 S L17 NOT L16
L19            11 S L18 AND (FLAVIVIR? OR DENG?)
                E DUBOIS D R/AU
L20            31 S E3

=> s l20 not (l12 or l17)
L21             9 L20 NOT (L12 OR L17)

=> d l21,cbib,ab,1-9

L21  ANSWER 1 OF 9     MEDLINE on STN
95289547.    PubMed ID: 7771614.   Evaluation of the severe combined
     immunodeficient (SCID) mouse as an animal model for dengue viral
     infection. Wu S J; Hayes C G; Dubois D R; Windheuser M G; Kang Y H;
     Watts D M; Sieckmann D G. (Infectious Diseases Department, Naval Medical
     Research Institute, Bethesda, MD 20889-5607, USA. ) The American journal
     of tropical medicine and hygiene, (1995 May) Vol. 52, No. 5, pp. 468-76.
     Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States.
     Language: English.
AB   Severe combined immunodeficient (SCID) mice reconstituted with human
     peripheral blood lymphocytes (hu-PBL) were evaluated as an animal model
     for demonstrating dengue (DEN) viral infection.  Reconstituted mice
     (hu-PBL-SCID) that demonstrated successful engraftment by the presence of
     serum titers of human immunoglobulin (Ig) were inoculated
     intraperitoneally with DEN virus serotype 1 (DEN-1).  Serial blood samples
     were taken postinoculation and assayed for virus in C6/36 cells.  The
     identity of all viral isolates was confirmed by an immunofluorescence
     antibody assay using DEN-1 monoclonal antibody.  A total of six
     experiments were performed using different procedures of reconstitution
     and infection, and in three of these experiments, DEN-1 virus was
     recovered from the hu-PBL-SCID mice.  In the first successful experiment,
     DEN-1 virus was recovered on postinoculation day (PID) 24 from blood,
     spleen, thymus, and lung tissues of one of eight hu-PBL-SCID mice.  A
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monocytes infected in vitro with DEN-1 virus. Virus was recovered from the blood of mice between PID 15 and 23, and from lung tissue of one of these mice. In a third experiment, seven SCID mice were treated initially with anti-asialo GM1 antibody to eliminate natural killer cells, and then were injected simultaneously with a mixture of hu-PBL and DEN-1 virus. Virus was demonstrated in the blood of one mouse on PID 38, and in another mouse on PID 8, 12, 20, 24, and 36.(ABSTRACT TRUNCATED AT 250 WORDS)

L21 ANSWER 2 OF 9 MEDLINE on STN

93277372. PubMed ID: 8503779. Comparison of replication rates and pathogenicities between the SA14 parent and SA14-14-2 vaccine strains of Japanese encephalitis virus in mouse brain neurons. Hase T; **Dubois D R**; Summers P L; Downs M B; Ussery M A. (Department of Ultrastructural Pathology, Walter Reed Army Institute of Research, Washington, D.C.) Archives of virology, (1993) Vol. 130, No. 1-2, pp. 131-43. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB The replication rates and pathogenicities of the SA 14 parent and SA 14-14-2 vaccine strains of Japanese encephalitis (JE) virus in neurons of the mouse brain following intracerebral inoculation were compared. All the mice inoculated with the SA 14 parent strain died within one week postinoculation (p.i.), whereas all the mice inoculated with the SA 14-14-2 vaccine strains survived without showing any signs of central nervous system (CNS) involvement. The virus titers of the mouse brains inoculated with the SA 14 strain reached progressively higher levels until day 5 when the animals died. On the other hand, the virus titers of the mouse brains inoculated with the SA 14-14-2 strain persisted at low levels for several days and could not be detected after 10 days. In the routine electron microscopical study, a majority of neurons in the mouse brains inoculated with the SA 14 strain contained virions and showed characteristic cytopathological changes in connection with viral replication. In the brains inoculated with the SA 14-14-2 strain, however, we failed to find neurons containing virions or showing characteristic cytopathological changes. In the alkaline phosphatase immunostaining of paraffin-embedded sections, a majority of neurons in the brains of mice inoculated with the SA 14 strain stained positively on day 5 p.i., but only a small number of neurons in scattered small foci stained positively in the brains inoculated with the SA 14-14-2 strain. The immunogold staining of Vibratome sections also revealed the identical patterns; moreover, electron microscopical examination of the immunopositive foci of the brain inoculated with the vaccine strain revealed neurons that contained virions in dilated cisternae of rough endoplasmic reticulum (RER), indicating that the SA 14-14-2 strain also replicated, albeit poorly, in neurons. The present results showed that upon intracerebral inoculation into mice the SA 14 parent strain of JE virus grew vigorously in a large number of neurons, killing the animals, while the SA 14-14-2 vaccine strain grew poorly only in a small number of neurons without causing mortality. Possible mechanisms involved in the alteration of pathogenicity between the SA 14 parent virus and the SA 14-14-2 vaccine virus are discussed.

L21 ANSWER 3 OF 9 MEDLINE on STN

93228183. PubMed ID: 8385887. Increased immunogenicity and protective efficacy in outbred and inbred mice by strategic carboxyl-terminal truncation of Japanese encephalitis virus envelope glycoprotein. Jan L R; Yang C S; Henchal L S; Sumiyoshi H; Summers P L; **Dubois D R**; Lai C J. (Molecular Viral Biology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.) The American journal of tropical medicine and hygiene, (1993 Mar) Vol. 48, No. 3, pp. 412-23. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB We constructed recombinant vaccinia viruses expressing the full-length envelope (E) glycoprotein of Japanese encephalitis virus (JEV) or a strategically truncated E glycoprotein, approximately 80% of the N-terminal sequence, and compared their antigenic structure and protective immunity in mice. The truncation site in the JEV E glycoprotein sequence corresponds to the position that had been shown to increase the immunogenicity of dengue type 4 or type 2 virus E glycoprotein. Analysis of the JEV E glycoprotein in recombinant virus-infected cells showed that C-terminally truncated E retains an antigenic structure similar to that of the full-length E glycoprotein. The full-length JEV E glycoprotein was detected predominantly intracellularly, while a small fraction (< 2%) was present on the cell surface. On the other hand, the truncated 80% E glycoprotein exhibited an alteration in the intracellular transport pathway resulting in increased accumulation (10-25%) on the cell surface and secretion (6-10%) into the medium. The C-terminally truncated E glycoprotein induced a greater antibody response and a higher level of protective immunity than did the full-length E glycoprotein in outbred CD-1 mice as well as in two strains of inbred mice that differ in their resistance to intraperitoneal (ip) JEV infection. In the case of outbred CD-1 and inbred C57/Bl mice, which possess a dominant autosomal genetic locus that controls resistance to a high dose of ip infection of JEV or the capacity to acquire resistance to intracerebral JEV infection,

antibodies.

L21 ANSWER 4 OF 9 MEDLINE on STN

91120043. PubMed ID: 2177623. Comparative study of mouse brains infected with Japanese encephalitis virus by intracerebral or intraperitoneal inoculation. Hase T; **Dubois D R**; Summers P L. (Department of Ultrastructural Pathology, Walter Reed Army Institute of Research, Washington, DC 20307.) International journal of experimental pathology, (1990 Dec) Vol. 71, No. 6, pp. 857-69. Journal code: 9014042. ISSN: 0959-9673. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The brains of mice infected with Japanese encephalitis (JE) virus by intracerebral inoculation (IC), intraperitoneal inoculation with sham intracerebral inoculation (IP+sIC), and intraperitoneal inoculation (IP) were studied by light and electron microscopy. The mortality rates and mean survival days were 100% and 4.8 days for the IC group, 92% and 9.0 days for the IP+sIC group, and 58% and 13.4 days for the IP group. Accordingly, the brain samples of sick mice were examined by light and electron microscopy 4 days post-inoculation (p.i.) for the IC group, 7 days p.i. for the IP+sIC group and 12 days p.i. for the IP group. In light microscopy, the mouse brains in the IC group showed little inflammatory change with only mild generalized glial-cell proliferation and mononuclear cell infiltration. In electron microscopy, however, a majority of neurons in the brain were seen to be infected with virus that replicated exclusively in the neuronal secretory system, including rough endoplasmic reticulum (RER) and the Golgi apparatus. In contrast, light microscopic observation of the brains from the IP+sIC and the IP groups showed prominent inflammatory changes with leucocytic infiltration and perivascular cuffing. Neuronal degeneration and neuronophagia were also prominent. In electron microscopy, neurons were infected in the same manner as in the IC group, but showed more advanced degenerative changes with marked cytoplasmic rarefaction and frequent neuronal disintegration. Mononuclear cells were frequently found in direct contact with degenerating and disintegrating neurons. The results showed that (a) the basic process of JE virus replication in brain neurons was present in the three groups of mice, (b) in the peripherally inoculated mice the process was accompanied by inflammatory reaction with resultant neuronal destruction, and (c) breach in the blood-brain barrier at the time of peripheral viral inoculation played an important role in the viral invasion of the CNS.

L21 ANSWER 5 OF 9 MEDLINE on STN

90381202. PubMed ID: 2169298. Ultrastructural changes of mouse brain neurons infected with Japanese encephalitis virus. Hase T; Summers P L; **Dubois D R**. (Department of Ultrastructural Pathology, Walter Reed Army Institute of Research, Washington, DC 20307.) International journal of experimental pathology, (1990 Aug) Vol. 71, No. 4, pp. 493-505. Journal code: 9014042. ISSN: 0959-9673. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Ultrastructural changes of mouse brain neurons infected intracerebrally with Japanese encephalitis (JE) virus were studied. JE virus selectively infected the neurons, causing ultrastructural changes in association with viral replication in the cellular secretory system, principally involving rough endoplasmic reticulum (RER) and the Golgi apparatus. In the early phase of infection, RER of infected neurons showed hypertrophic changes, containing assembling virions within its dilated cisternae. In the later phase, the RER became cystic and degenerative and dissolved into the cytoplasm. The Golgi apparatus also contained in its saccules multiple virions, presumably transported from the RER cisternae, which were then released into the cytoplasm within coated vesicles for secretory-type exocytosis. In the process, the Golgi apparatus also fragmented and degenerated through vesiculation, vacuolation, and dispersion. Thus, the JE virus infection of neurons resulted in obliteration of RER and the Golgi apparatus, leaving behind the rarefied cytoplasm devoid of these organelles. However, destruction of the neurons themselves was not prominent and infected neurons in the later phase of infection showed some regenerative changes of these membranous organelles. The cause of death of infected animals, therefore, appeared to be extensive neuronal dysfunction rather than neuronal destruction in the CNS.

L21 ANSWER 6 OF 9 MEDLINE on STN

79212106. PubMed ID: 378507. Infectious mononucleosis associated with fatal beta hemolytic streptococcal infection. **DuBois D R**; Baehner R L. Clinical pediatrics, (1979 Aug) Vol. 18, No. 8, pp. 511-2. Journal code: 0372606. ISSN: 0009-9228. Pub. country: United States. Language: English.

L21 ANSWER 7 OF 9 MEDLINE on STN

78109547. PubMed ID: 415061. Cultivation of dengue virus type 2 in candidate substrates for vaccine production. **Dubois D R**; Berman S; Rourke S M; Timchak R L; Lowenthal J P. Journal of biological standardization, (1978 Jan) Vol. 6, No. 1, pp. 21-6. Journal code: 0400335. ISSN: 0092-1157. Pub. country: ENGLAND: United Kingdom. Language: English.

L21 ANSWER 8 OF 9 MEDLINE on STN
75154249. PubMed ID: 1039461. Indications of an unhealthy relationship between parents and premature infant. **Dubois D R**. JOGN nursing; journal of obstetric, gynecologic, and neonatal nursing, (1975 May-Jun) Vol. 4, No. 3, pp. 21-4. Journal code: 0347421. ISSN: 0090-0311. Pub. country: United States. Language: English.

L21 ANSWER 9 OF 9 MEDLINE on STN
72201964. PubMed ID: 4555634. Preparation of purified suspensions of Coxiella burneti by Genetron extraction followed by continuous-flow ultracentrifugation. **Dubois D R**; Cutchins E C; Berman S; Lowenthal J P; Timchak R L. Applied microbiology, (1972 May) Vol. 23, No. 5, pp. 841-5. Journal code: 7605802. ISSN: 0003-6919. Pub. country: United States. Language: English.

=> d his

(FILE 'HOME' ENTERED AT 11:02:05 ON 27 JUN 2006)

FILE 'USPATFULL' ENTERED AT 11:02:14 ON 27 JUN 2006
E ECKELS KENNETH/IN
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L3 0 S L1 NOT L1
E DUBOIS DORIA R/IN
L4 6 S E3
L5 0 S L4 NOT L1

FILE 'WPIDS' ENTERED AT 11:03:30 ON 27 JUN 2006
E ECKELS K/IN
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E DUBOIS D R/IN
L9 29 S E3
L10 20 S L9 NOT (L6 OR L8)
L11 1 S L10 AND (DEN? OR FLAVIVIR?)

FILE 'MEDLINE' ENTERED AT 11:05:22 ON 27 JUN 2006
E ECKELS K/AU
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L13 50 S L12 AND (FLAVIVIR? OR DEN?)
L14 42 S L13 AND VACCIN?
L15 1 S L14 AND (MULTIVALENT)
L16 41 S L14 NOT L15
E PUTNAK J R/AU
L17 21 S E3-E4
L18 17 S L17 NOT L16
L19 11 S L18 AND (FLAVIVIR? OR DENG?)
E DUBOIS D R/AU
L20 31 S E3
L21 9 S L20 NOT (L12 OR L17)

=> s (dengue virus)
5171 DENGUE
415203 VIRUS
L22 2919 (DENGUE VIRUS)
(DENGUE(W)VIRUS)

=> s l22 and (45AZ5 or S16803 or CH53489 or 341750)
6 45AZ5
2 S16803
5 CH53489
6 341750
L23 13 L22 AND (45AZ5 OR S16803 OR CH53489 OR 341750)

=> s l23 not (l12 or l16)
L24 5 L23 NOT (L12 OR L16)

=> d l24,cbib,ab,1-5

L24 ANSWER 1 OF 5 MEDLINE on STN
2004040700. PubMed ID: 14740949. Biologic properties of dengue viruses following serial passage in primary dog kidney cells: studies at the University of Hawaii. Halstead Scott B; Marchette Nyven J. (Department of Tropical Medicine and Medical Microbiology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii, USA.. halsteads@erols.com) . The American journal of tropical medicine and hygiene, (2003 Dec) Vol. 69, No. 6 Suppl, pp. 5-11. Journal code:

AB Serial passage at low dilution of seven different wild-type dengue (DEN) viruses into primary dog kidney (PDK) cell cultures placed selective pressure that resulted in the following changes from parental phenotype: smaller plaques in LLC-MK2 cells, absent plaque formation in green monkey kidney cells, lack of a cytopathic effect in LLC-MK2 cells, shut-off of virus replication at high temperatures (temperature sensitivity), reduced virulence for rhesus monkeys manifested by reduced or absent viremia and/or absence of a secondary-type antibody response following homotypic challenge, and progressive increase in the mean day of death following intracerebral inoculation of sucking mice. Two DEN-1 strains showed most of these changes by the 15th PDK passage. Only one of two DEN-2 strains studied was carried to the 50th PDK passage at the University of Hawaii. For the latter strain, both the temperature of viral replicative shutoff and mouse neurovirulence were reduced. Three DEN-4 strains showed similar late-passage biologic marker changes. The observations made, although not exhaustive, provide laboratory correlates for virus strains that have shown reduced virulence but retained immunogenicity in humans. Candidate human vaccines have been prepared from five of the studied strains: DEN-1 (16007) at PDK 13, DEN-2 (16681 and S-16803) at PDK 50 or above, and DEN-4 (1036 and **341750**) at PDK 48 and 20, respectively.

L24 ANSWER 2 OF 5 MEDLINE on STN

2001351182. PubMed ID: 11414440. Highly conserved nucleotide sequence and its deduced amino acids of the 5'-noncoding region and the capsid protein of a Bangkok isolate dengue-3 virus. Attatippaholkun W H; Attatippaholkun M K; Nisalak A; Vaughn D W; Innis B L. (Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.) The Southeast Asian journal of tropical medicine and public health, (2000) Vol. 31 Suppl 1, pp. 119-25. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.

AB The dengue-3 virus genome encodes an uninterrupted open reading frame (ORF) flanked by 5' and 3' non-coding regions. The order of proteins encoded in dengue-3 virus ORF, as with other flaviviruses, is: Cap 5'-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-3'. The nucleotide sequence of the 5'-noncoding region and the capsid protein of dengue-3virus (a Bangkok isolate: **CH53489** isolated by USAMC-AFRIMS in 1973) has been analyzed in both forward and reverse directions. The PCR-based cycle sequencing technique by the enzymatic method of Sanger et al (1977) using a sequencing primer 5'-end labeled with gamma32P-ATP is the method of our choice for sequencing analysis. One cDNA template was prepared by RT-PCR technique starting from the 5'-end nucleotide 1-465 of the dengue-3 genome. In our cycle sequencing experiments, the substitution of 7-deaza-dG was used for dG in DNA eliminated much of the secondary structures that produced gel artifacts. The final sequence result of this cDNA template was established from its sequence data determined on both strands in opposite directions. Alignment between the newly established nucleotide sequence as well as its deduced amino acid sequence of the Bangkok dengue-3 virus and the published sequence data of the dengue-3 prototype (H87) was manipulated by the PC-DOS-GIBIO-DNASIS TM 06-00 (Hitachi Software). According to the deduced amino acid sequence of the Bangkok dengue-3 virus, its C protein was found to be highly positively charged because of large numbers of lysine and arginine. The homology of the nucleotide sequence between the two dengue-3 virus revealed 97%. The deduced amino acid sequences from the nucleotides 95-465 of the two viruses showed the same indicating highly conserved capsid proteins. Multiple alignment of the nucleotide sequences as well as the deduced amino acid sequences among the Bangkok dengue-3 virus and other dengue 3 viruses also confirmed the highly conserved 5'-noncoding regions and the capsid proteins.

L24 ANSWER 3 OF 5 MEDLINE on STN

2001237529. PubMed ID: 11304057. Limited potential for transmission of live **dengue virus** vaccine candidates by Aedes aegypti and Aedes albopictus. Sardelis M R; Edelman R; Klein T A; Innis B L; Putnak J R; Jones J W; Turell M J. (Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011, USA.) The American journal of tropical medicine and hygiene, (2000 Jun) Vol. 62, No. 6, pp. 698-701. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB To evaluate the transmission risk of four live dengue (DEN) vaccine candidates developed by the U.S. Army (DEN-1, **45AZ5** PDK 20; DEN-2, **S16803** PDK 50; DEN-3, **CH53489** PDK 20; and DEN-4, **341750** PDK 20), we tested 3,010 Aedes aegypti and 1,576 Aedes albopictus mosquitoes blood-fed on 21 volunteers who had been administered one of the four vaccine candidates or the licensed yellow fever (YF) vaccine (17D). We used an indirect immunofluorescence assay (IFA) to detect DEN or YF viral antigen in the heads of mosquitoes. Corresponding to the lack of a detectable viremia among volunteers inoculated 8-13 days previously with live DEN-1 or DEN-2 vaccine candidates, only six mosquitoes developed disseminated infections after feeding on these volunteers. These six mosquitoes included 4 of 247 Ae. albopictus fed on volunteers inoculated with the DEN-1 vaccine candidate and 2 of 528 Ae. aegypti fed on volunteers

each of these IFA-positive mosquitoes by isolating infectious virus from the mosquito's body in Vero-cell culture. None of the 1,252 or the 969 mosquitoes fed on DEN-3 or DEN-4 recipients, respectively, were infected. Overall, dissemination rates in Ae. albopictus and Ae. aegypti were low. Dissemination rates were 0.5%, 0.3%, < 0.1%, and < 0.1% for the DEN-1 through DEN-4 vaccine candidates, respectively. Because of the observed low dissemination rates, it is unlikely that these vaccine viruses would be transmitted under natural conditions.

L24 ANSWER 4 OF 5 MEDLINE on STN
1999101219. PubMed ID: 9886128. Nucleotide sequence and deduced amino acid sequence of the nonstructural proteins of dengue type 3 virus, Bangkok genotype. Attatippaholkun W H; Attatippaholkun M K; Nisalak A; Vaughn D W; Innis B L. (Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand.) The Southeast Asian journal of tropical medicine and public health, (1998 Jun) Vol. 29, No. 2, pp. 361-6. Journal code: 0266303. ISSN: 0125-1562. Pub. country: Thailand. Language: English.
AB The nucleotide sequence of the nonstructural protein gene (1,610 bases) of dengue 3 virus (Bangkok genotype; **CH53489** isolated in 1973) has been determined in both forward and reverse directions. The PCR based cycle sequencing technic by the enzymatic method of Sanger et al using a sequencing primer 5'-end labeled with gamma-32P-ATP was the method of our choice for sequence analysis. Two cDNA templates were prepared by RT-PCR technique starting from the nucleotides 6,306-6,969 and 6,925-7,915 of the dengue 3 genome with the lengths of 663 and 990 base pairs respectively. In our cycle sequencing experiments, it has been observed that the substitution of 7-deaza-dG for dG in DNA eliminated most of the secondary structures that produce gel artifacts. The final sequence results of these two cDNA templates were established from their sequence data determined on both strands in opposite directions. Alignment between the newly established nucleotide sequences as well as their deduced amino acid sequences of the Bangkok dengue 3 (**CH53489**) virus and the published sequence data of the dengue 3 prototype (H87) was manipulated by the PC-DOS-GIBIO DNASIS TM 06-00 software. The homology of the nucleotide sequences between the two dengue 3 viruses was 96.65%. The deduced amino acid sequence from nucleotides 6,306-7,915 of the two viruses showed conserved amino acids of the nonstructural protein NS4a and 6 amino acid changes in NS4b and NS5.

L24 ANSWER 5 OF 5 MEDLINE on STN
87290443. PubMed ID: 3615798. **Dengue virus** vaccine studies in Puerto Rico: a review. Kraiselburd E. Puerto Rico health sciences journal, (1987 Apr) Vol. 6, No. 1, pp. 27-9. Journal code: 8303541. ISSN: 0738-0658. Pub. country: Puerto Rico. Language: English.
AB This review summarizes part of the work performed at the Virology Laboratories (Department of Microbiology, University of Puerto Rico School of Medicine) with live attenuated **dengue virus** vaccines obtained from the Walter Reed Army Institute of Research. Vaccines were tested for their respective immunogenicity and attenuation in rhesus monkeys (Maccaca mulatta) and in mosquitoes (Toxorynchites amboinensis), respectively. This experimental model revealed that out of 6 vaccines tested, only two (DEN-2/S-1 and DEN-4 strain **341750**) should be further evaluated for safety and immunogenicity. Further studies are required to develop an effective vaccine to prevent dengue fever and its hemorrhagic manifestations.

=> d his

(FILE 'HOME' ENTERED AT 11:02:05 ON 27 JUN 2006)

FILE 'USPATFULL' ENTERED AT 11:02:14 ON 27 JUN 2006
E ECKELS KENNETH/IN
L1 11 S E3-E4
E PUTNAK JOSEPH R/IN
L2 9 S E2-E3
L3 0 S L1 NOT L1
E DUBOIS DORIA R/IN
L4 6 S E3
L5 0 S L4 NOT L1

FILE 'WPIDS' ENTERED AT 11:03:30 ON 27 JUN 2006
E ECKELS K/IN
L6 13 S E3 OR E4
E PUTNAK J R/IN
L7 12 S E3
L8 3 S L7 NOT L6
E DUBOIS D R/IN
L9 29 S E3
L10 20 S L9 NOT (L6 OR L8)
L11 1 S L10 AND (DEN? OR FLAVIVIR?)

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L12           64 S E3-E5
L13           50 S L12 AND (FLAVIVIR? OR DEN?)
L14           42 S L13 AND VACCIN?
L15            1 S L14 AND (MULTIVALENT)
L16           41 S L14 NOT L15
                E PUTNAK J R/AU
L17           21 S E3-E4
L18           17 S L17 NOT L16
L19           11 S L18 AND (FLAVIVIR? OR DENG?)
                E DUBOIS D R/AU
L20           31 S E3
L21            9 S L20 NOT (L12 OR L17)
L22         2919 S (DENGUE VIRUS)
L23           13 S L22 AND (45AZ5 OR S16803 OR CH53489 OR 341750)
L24            5 S L23 NOT (L12 OR L16)

=> s l22 and vaccin?
        167376 VACCIN?
L25        418 L22 AND VACCIN?

=> s l25 and multivalent
          2699 MULTIVALENT
L26          6 L25 AND MULTIVALENT

=> d l26,cbib,ab,1-6

L26  ANSWER 1 OF 6     MEDLINE on STN
2005675224.     PubMed ID: 16125280.   Tetravalent neutralizing antibody
     response against four dengue serotypes by a single chimeric dengue
     envelope antigen. Apt Doris; Raviprakash Kanakatte; Brinkman Alice;
     Semyonov Andrey; Yang Shumin; Skinner Craig; Diehl Lori; Lyons Richard;
     Porter Kevin; Punnonen Juha. (Maxygen Inc., Human Therapeutics, 515
     Galveston Drive, Redwood City, CA 94063, USA.. doris.apt@maxygen.com) .
     Vaccine, (2006 Jan 16) Vol. 24, No. 3, pp. 335-44.  Electronic
     Publication: 2005-08-10. Journal code: 8406899. ISSN: 0264-410X. Pub.
     country: Netherlands. Language: English.
AB   We employed DNA shuffling and screening technologies to develop a single
     recombinant dengue envelope (E) antigen capable of inducing neutralizing
     antibodies against all four antigenically distinct dengue serotypes.  By
     DNA shuffling of codon-optimized dengue 1-4 E genes, we created a panel of
     novel chimeric clones expressing C-terminal truncated E antigens that
     combined epitopes from all four dengue serotypes.  DNA vaccines encoding
     these novel chimeras induced multivalent T cell and neutralizing
     antibody responses against all four dengue serotypes in mice.  By
     contrast, a mixture of four unshuffled, parental DNA vaccines failed to
     produce tetravalent neutralizing antibodies in mice.  The neutralizing
     antibody titers for some of these antigens could be further improved by
     extending the sequences to express full-length pre-membrane and envelope
     proteins.  The chimeric antigens also protected mice against a lethal
     dengue-2 virus challenge.  These data demonstrate that DNA shuffling and
     associated screening can lead to the selection of multi-epitope antigens
     against closely related dengue virus serotypes and suggest a broad
     utility for these technologies in optimizing vaccine antigens.

L26  ANSWER 2 OF 6     MEDLINE on STN
2004629606.     PubMed ID: 15603884.   Dengue virus: molecular basis of cell
     entry and pathogenesis, 25-27 June 2003, Vienna, Austria. Halstead Scott
     B; Heinz Franz X; Barrett A D T; Roehrig John T. (Pediatric Dengue Vaccine
     Initiative, 5824 Edson Lane, Rockville, MD 20852, USA..
     halsteads@erols.com) . Vaccine, (2005 Jan 4) Vol. 23, No. 7, pp. 849-56.
     Journal code: 8406899. ISSN: 0264-410X. Pub. country: Netherlands.
     Language: English.
AB   Multivalent dengue vaccines now in late stage development pose unique
     vaccine safety challenges in that primary or secondary vaccine
     failures might place vaccines at risk to antibody-dependent enhanced
     (ADE) wild-type dengue infections.  This conference was organized to
     address this unique vaccine safety issue.  New data were presented on
     the structure of dengue and other flaviviruses, the cellular receptors of
     dengue virus for biologically relevant cells, dengue viral cell entry
     mechanisms and mechanisms underlying in vivo protection, neutralization
     and enhancement of dengue virus infection.  It was concluded that a
     targeted research program should aim to develop an in vitro test to
     characterize persons immunized with dengue vaccines as completely or
     partially protected.  Achievement of this aim will require a better
     understanding of the basic mechanisms by which dengue viruses recognize,
     attach, enter and infect relevant human cells and how antibodies protect
     against dengue infections.

L26  ANSWER 3 OF 6     MEDLINE on STN
2001222564.     PubMed ID: 11312014.   Safety and immunogenicity of attenuated
```

Kanesa-thasan N; Sun W; Kim-Ahn G; Van Albert S; Putnak J R; King A; Raengsakulsrach B; Christ-Schmidt H; Gilson K; Zahradnik J M; Vaughn D W; Innis B L; Saluzzo J F; Hoke C H Jr. (Walter Reed Army Institute of Research, Washington, DC, USA.. niranjan.kanesa-thasan@na.amedd.army.mil) . Vaccine, (2001 Apr 30) Vol. 19, No. 23-24, pp. 3179-88. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.

AB A randomized, controlled, double-blinded study was conducted to determine safety and immunogenicity of five live attenuated dengue **vaccines** produced by Aventis Pasteur (AvP). The study was completed with 40 flavivirus non-immune volunteers: five recipients of each monovalent (dengue-1, dengue-2, dengue-3, or dengue-4) **vaccine**, ten recipients of tetravalent (dengue-1, dengue-2, dengue-3, and dengue-4) **vaccine**, and ten recipients of **vaccine** vehicle alone. All **vaccines** were administered in a single subcutaneous dose (range, 3.6-4.4 log(10) plaque forming units). No serious adverse reactions occurred in volunteers followed for 6 months after **vaccination**. Five **vaccine** recipients developed fever (T > or = 38.0 degrees C), including four tetravalent **vaccinees** between days 8 and 10 after **vaccination**. Dengue-1, dengue-2, dengue-3, or dengue-4 **vaccine** recipients reported similar frequency of mild symptoms of headache, malaise, and eye pain. Tetravalent **vaccinees** noted more moderate symptoms with onset from study days 8-11 and developed maculopapular rashes distributed over trunk and extremities. Transient neutropenia (white blood cells < 4000/mm3) was noted after **vaccination** but not thrombocytopenia (platelets < 100,000/mm3). All dengue-3, dengue-4, and tetravalent **vaccine** recipients were viremic between days 7 and 12 but viremia was rarely detected in dengue-1 or dengue-2 **vaccinees**. All dengue-2, dengue-3, and dengue-4, and 60% of dengue-1 **vaccine** recipients developed neutralizing and/or immunoglobulin M antibodies. All tetravalent **vaccine** recipients were viremic with dengue-3 virus and developed neutralizing antibodies to dengue-3 virus. Seven volunteers also had **multivalent** antibody responses, yet the highest antibody titers were against dengue-3 virus. The AvP live attenuated **dengue virus vaccines** are safe and tolerable in humans. The live attenuated tetravalent dengue **vaccine** was most reactogenic, and preferential replication of dengue-3 virus may have affected its infectivity and immunogenicity.

L26 ANSWER 4 OF 6 MEDLINE on STN

1998033180. PubMed ID: 9367357. Analysis of a recombinant dengue-2 virus-dengue-3 virus hybrid envelope protein expressed in a secretory baculovirus system. Bielefeldt-Ohmann H; Beasley D W; Fitzpatrick D R; Aaskov J G. (Centre for Molecular Biotechnology, School of Life Science, Queensland University of Technology, Brisbane, Australia.. helle@biosci.uq.edu.au) . The Journal of general virology, (1997 Nov) Vol. 78 (Pt 11), pp. 2723-33. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In a step towards a tetravalent **dengue virus** subunit **vaccine** which is economical to produce, highly immunogenic and stable, a hybrid **dengue virus** envelope (E) protein molecule has been constructed. It consists of 36 amino acids from the membrane protein, the N-terminal 288 amino acids of the dengue-2 virus E protein plus amino acids 289-424 of the dengue-3 virus E protein. It has been engineered for secretory expression by fusion to a mellitin secretory signal sequence and truncation of the hydrophobic transmembrane segment. Using the baculovirus expression system and serum-free conditions, more than 95% of recombinant dengue-2 virus-dengue-3 virus hybrid E protein (rD2D3E) was secreted into the cell culture supernatant in a stable form with multiple features indicative of preserved conformation. The hybrid molecule reacted with a panel of **dengue virus**- and flavivirus-specific MAbs which recognize linear or conformational epitopes on dengue virions. Human **dengue virus**-specific antisera also reacted with the protein. The hybrid rD2D3E protein was able to inhibit the in vitro binding of dengue-2 and dengue-3 viruses to human myelomonocytic cells, suggesting that the receptor-binding epitope(s) was preserved. Adjuvant-free immunization with the hybrid protein induced an antibody response to both dengue-2 and dengue-3 virus in outbred mice, comparable in strength to that of individual rD2E and rD3E proteins. Notably, these antibody responses were primarily of the IgG2a and IgG2b isotype. A strong **dengue virus** cross-reactive T cell response was also induced in the mice, suggesting that **dengue virus** hybrid E proteins could form the basis of an efficacious **multivalent dengue virus vaccine**.

L26 ANSWER 5 OF 6 MEDLINE on STN

96215657. PubMed ID: 8645110. Immunisation with DNA polynucleotides protects mice against lethal challenge with St. Louis encephalitis virus. Phillpotts R J; Venugopal K; Brooks T. (Microbiology Group, Chemical and Biological Defence Establishment, Porton Down, Wiltshire, U.K.) Archives of virology, (1996) Vol. 141, No. 3-4, pp. 743-9. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB In vivo transfection by intramuscular injection with plasmids expressing the immunogenic proteins of microbial pathogens has considerable potential

animals. Here we report that weanling mice given a single intramuscular injection of 50 micrograms of a plasmid, pSLE1 expressing the St. Louis encephalitis virus (SLE) prM/E protein under the control of the cytomegalovirus immediate early protein promoter produced SLE-specific antibody and were protected against lethal challenge with the virulent virus. Polynucleotide **vaccine** technology provides a unique opportunity to produce **vaccines** against flavivirus diseases of low incidence cheaply and rapidly, and to produce **multivalent vaccines** such as would be required for immunisation against **dengue virus** disease.

L26 ANSWER 6 OF 6 MEDLINE on STN
93381798. PubMed ID: 8371350. **Dengue virus**-specific human CD4+ T-lymphocyte responses in a recipient of an experimental live-attenuated **dengue virus** type 1 **vaccine**: bulk culture proliferation, clonal analysis, and precursor frequency determination. Green S; Kurane I; Edelman R; Tacket C O; Eckels K H; Vaughn D W; Hoke C H Jr; Ennis F A. (Department of Medicine, University of Massachusetts Medical Center, Worcester 01655.) Journal of virology, (1993 Oct) Vol. 67, No. 10, pp. 5962-7. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We analyzed the CD4+ T-lymphocyte responses to dengue, West Nile, and yellow fever viruses 4 months after immunization of a volunteer with an experimental live-attenuated **dengue virus** type 1 **vaccine** (DEN-1 45AZ5). We examined bulk culture proliferation to noninfectious antigens, determined the precursor frequency of specific CD4+ T cells by limiting dilution, and established and analyzed CD4+ T-cell clones. Bulk culture proliferation was predominantly **dengue virus** type 1 specific with a lesser degree of cross-reactive responses to other **dengue virus** serotypes, West Nile virus, and yellow fever virus. Precursor frequency determination by limiting dilution in the presence of noninfectious **dengue virus** antigens revealed a frequency of antigen-reactive cells of 1 in 1,686 peripheral blood mononuclear cells (PBMC) for **dengue virus** type 1, 1 in 9,870 PBMC for **dengue virus** type 3, 1 in 14,053 PBMC for **dengue virus** type 2, and 1 in 17,690 PBMC for **dengue virus** type 4. Seventeen CD4+ T-cell clones were then established by using infectious **dengue virus** type 1 as antigen. Two patterns of **dengue virus** specificity were found in these clones. Thirteen clones were **dengue virus** type 1 specific, and four clones recognized both **dengue virus** types 1 and 3. Analysis of human leukocyte antigen (HLA) restriction revealed that five clones are HLA-DRw52 restricted, one clone is HLA-DP3 restricted, and one clone is HLA-DP4 restricted. These results indicate that in this individual, the CD4+ T-lymphocyte responses to immunization with live-attenuated **dengue virus** type 1 **vaccine** are predominantly serotype specific and suggest that a **multivalent vaccine** may be necessary to elicit strong serotype-cross-reactive CD4+ T-lymphocyte responses in such individuals.

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COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              18.24     122.50

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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 27 Jun 2006 (20060627/PD)
FILE LAST UPDATED: 27 Jun 2006 (20060627/ED)
HIGHEST GRANTED PATENT NUMBER: US7069595
HIGHEST APPLICATION PUBLICATION NUMBER: US2006137066
CA INDEXING IS CURRENT THROUGH 27 Jun 2006 (20060627/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 27 Jun 2006 (20060627/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Feb 2006
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Feb 2006


=> s (dengue)
L27         2786 (DENGUE)

=> s l27 and vaccin?
          50064 VACCIN?
L28         2091 L27 AND VACCIN?

=> s l28 and attenuate?
         117207 ATTENUATE?
L29         1107 L28 AND ATTENUATE?

=> s l29 and multivalent
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L30          179 L29 AND MULTIVALENT

=> s l30 and ay<2001
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L31 54 L30 AND AY<2001

=> s l31 and dengue/clm
284 DENGUE/CLM
L32 14 L31 AND DENGUE/CLM

=> d l32,cbib,clm,1-14

L32 ANSWER 1 OF 14 USPATFULL on STN
2004:146867 Recombinant dimeric envelope **vaccine** against flaviviral infection
.
Peters, Iain D., Bozeman, MT, United States
Coller, Beth-Ann G., Woluwe Saint Lambert, BELGIUM
McDonell, Michael, Bogart, GA, United States
Ivy, John M., College Station, TX, United States
Harada, Kent, Honolulu, HI, United States
Hawaii Biotechnology Group, Inc., Aiea, HI, United States (U.S. corporation)
US 6749857 B1 20040615
APPLICATION: US 1999-376463 19990818 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed:
1. A **vaccine** that generates a protective, neutralizing antibody response to a Flavivirus in a murine host, wherein said **vaccine** comprises a therapeutically effective amount of a dimeric 80%E, said dimeric 80%E having been secreted as a recombinantly produced protein from Drosophila Schneider cells, wherein 80%E represents the N-terminal 80% portion of the protein from residue 1 to residue 395.

2. The **vaccine** of claim 1 wherein said dimeric 80%E is selected from the group consisting of: linked 80%E dimer; 80%E ZipperI; 80%E ZipperII; and 80%E Bundle.

3. The **vaccine** of claim 2 wherein the linked 80%E dimer is a truncated envelope protein of serotype DEN-1.

4. The **vaccine** of claim 2 wherein the linked 80%E dimer is a truncated envelope protein of serotype DEN-2.

5. The **vaccine** of claim 1 wherein the linked 80%E dimer is a truncated envelope protein of serotype DEN-3.

6. The **vaccine** of claim 1 wherein the linked 80%E dimer is a truncated envelope protein of serotype DEN-4.

7. A **multivalent vaccine** that generates a protective, neutralizing antibody response to a Flavivirus in a murine host, wherein said **vaccine** comprises a therapeutically effective amount of a first dimeric 80%E product of one flaviviral serotype; a second dimeric 80%E product of a second flaviviral serotype; a third dimeric 80%E product of a third flaviral serotype; and a fourth dimeric 80%E product of a fourth flaviviral serotype; wherein all dimeric 80%E products have been secreted as recombinantly produced protein from a Drosophila Schneider cell, wherein 80%E is the N-terminal 80% of the protein from residue 1 to 395.

8. The **vaccine** of claim 7 wherein said dimeric 80%E products are envelope proteins of serotypes selected from the group consisting of: DEN-1; DEN-2; DEN-3; and DEN-4.

9. The **vaccine** of claim 1 wherein said Flavivirus is a **dengue** virus.

10. The **vaccine** of claim 2 wherein said Flavivirus is a **dengue** virus.

11. The **vaccine** of claim 7 wherein said Flavivirus is a **dengue** virus.

12. An immunogenic polypeptide comprising a dimeric 80%E, said dimeric 80%E having been secreted as a recombinantly produced protein from Drosophila Schneider cells, wherein 80%E represents the N-terminal 80% of the protein from residue 1 to residue 395.

13. The immunogenic polypeptide of claim 12 wherein said dimeric 80%E is selected from the group consisting of: linked 80%E dimer, 80%E ZipperI; 80%E ZipperII; and 80%E bundle.

14. The immunogenic polypeptide of claim 13 wherein the linked 80%E dimer is a truncated envelope protein which is at least one member selected from the group consisting of serotype DEN-1, serotype DEN-2, serotype DEN-3, and serotype DEN-4.

15. An immunogenic composition that generates a protective, neutralizing

immunogenic polypeptide defined in claim 12 and a physiologically acceptable carrier.

16. The immunogenic composition defined in claim 15 further comprising an adjuvant.

17. The immunogenic composition defined in claim 15 wherein said adjuvant is Iscomatrix.

18. The immunodiagnostic for the detection of Flavivirus comprising the immunogenic polypeptide defined in claim 12.

19. A **multivalent** immunodiagnostic for the detection of Flavivirus comprising at least two of the immunogenic polypeptides defined in claim 12 of at least two flaviviral serotypes.

20. An immunodiagnostic kit for the detection of Flavivirus in a test subject comprising a) the immunogenic polypeptide defined in claim 12; b) a suitable support phase coated with dimeric 80%E; and c) labeled antibodies immunoreactive to antibodies from said test subject.

21. An immunodiagnostic kit for the detection of Flavivirus in a test subject comprising a) the **multivalent** immunodiagnostic polypeptide defined in claim 19; b) a suitable support phase coated with dimeric 80%E; and c) labeled antibodies immunoreactive to antibodies from said test subject.

L32 ANSWER 2 OF 14 USPATFULL on STN
2003:285090 **Multivalent dengue** virus **vaccine**.
Eckels, Kenneth H., Rockville, MD, United States
Putnak, Joseph R., Silver Spring, MD, United States
Dubois, Doria R., Wheaton, MD, United States
Innis, Bruce L., Haverford, PA, United States
Hoke, Charles H., Columbia, MD, United States
Wellington, Sun, Rockville, MD, United States
Kanessa-thasan, Niranjan, Rockville, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6638514 B1 20031028
APPLICATION: US 2000-535117 20000324 (9)
PRIORITY: US 2000-181724P 20000211 (60)
US 1999-126313P 19990326 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An immunogenic composition comprising two or more **attenuated dengue** viruses selected from the group consisting of a **dengue**-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a **dengue**-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a **dengue**-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a **dengue**-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652, and a physiologically acceptable vehicle.

2. The immunogenic composition according to claim 1 which further comprises an adjuvant to enhance the immune response.

3. The immunogenic composition of claim 1, formulated in a dose of 10^2 to 10^6 PFU of **attenuated** virus.

4. A **multivalent** live **attenuated dengue** virus **vaccine** comprising any combination of **dengue** virus serotypes selected from the group consisting of: a **dengue**-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-20 having the ATCC accession number VR-2648, a **dengue**-2 (DEN-2) virus having the sequence of DEN-2 strain S 16803 PDK-50 having the ATCC accession number VR-2653, a **dengue**-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a **dengue**-4 (DEN-4) virus having the sequence of DEN-4 strain 341750 PDK-20 having the ATCC accession number VR-2652.

5. The **dengue** virus **vaccine** of claim 4 wherein said **dengue** virus is produced in vertebrate cells.

6. The **dengue** virus **vaccine** of claim 5 wherein said cells are Vero cells.

7. The **dengue** virus **vaccine** of claim 4 wherein said **dengue**-1 virus is in the amount of 10^2 to 10^7 pfu/ml, said **dengue**-2 virus

in the amount of 10^2 to 10^7 pfu, and said **dengue**-4 virus is in the amount of 10^2 to 10^7 pfu/ml.

8. The **dengue** virus **vaccine** of claim 7 wherein said **vaccine** is administered subcutaneously.

9. An immunogenic composition comprising two or more **attenuated dengue** virus chosen from the group consisting of a **dengue**-1 (DEN-1) virus having the sequence of DEN-1 sawn 45AZ5 PDK-27 having the ATCC accession number PTA4810, a **dengue**-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-S0 having the ATCC accession number VR-2653, a **dengue**-3 (DEN-3) virus having the sequence of DEN-3 strain CH153489 PDK-20 having the ATCC accession number VR-2647, and a **dengue**-4 (DEN4) virus having the sequence of DEN-4 strain 341750 PDK6 having the ATCC accession number PTA4811, and a physiologically acceptable vehicle.

10. A **multivalent** live **attenuated dengue** virus **vaccine** comprising any combination of **dengue** virus serotypes selected from the group consisting of: a **dengue**-1 (DEN-1) virus having the sequence of DEN-1 strain 45AZ5 PDK-27 having the ATCC accession number PTA4810, a **dengue**-2 (DEN-2) virus having the sequence of DEN-2 strain S16803 PDK-50 having the ATCC accession number VR-2653, a **dengue**-3 (DEN-3) virus having the sequence of DEN-3 strain CH53489 PDK-20 having the ATCC accession number VR-2647, and a **dengue**-4 (DEN-4) virus having the sequence of DEN-4 stain 341750 PDK-6 having the ATCC accession number PTA-4811.

11. The **vaccine** of claim 10 wherein at least one virus os DEN-1 strain 45Az5 PDK-27 having the ATCC accessior number PTA-4810.

12. The **vaccine** of claim 10 wherein at least one virus is DEN-4 strain 341750 PDK-6 having the ATCC accessior number PTA-4811.

L32 ANSWER 3 OF 14 USPATFULL on STN
2003:155723 Polynucleotides encoding flavivirus and alphavirus **multivalent** antigenic polypeptides.
Punnonen, Juha, Palo Alto, CA, United States
Bass, Steven H., Hillsborough, CA, United States
Whalen, Robert Gerald, Paris, FRANCE
Howard, Russell, Los Altos Hills, CA, United States
Stemmer, Willem P. C., Los Gatos, CA, United States
Maxygen, Inc., Redwood City, CA, United States (U.S. corporation)
US 6576757 B1 20030610
APPLICATION: US 2000-724852 20001128 (9)
PRIORITY: US 1998-105509P 19981023 (60)
US 1998-74294P 19980211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A nucleic acid comprising a polynucleotide sequence encoding a recombinant **multivalent** antigenic polypeptide that comprises multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, wherein the recombinant **multivalent** antigenic polypeptide induces an immune response against the first and second antigenic polypeptides that is greater than the immune response induced by any one of the fist and second antigenic polypeptides against any other of the first and second antigenic polypeptides.

2. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide comprises multiple non-contiguous subsequences of at least a third antigenic polypeptide of at least a third flavivirus or alphavirus.

3. The nucleic acid of claim 1, wherein at least the first and second antigenic polypeptides are from a virus selected from the group consisting of a Venezuelan equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus complex, a virus of the tick-borne encephalitis virus complex, a **Dengue** virus, a yellow fever virus, a St. Louis encephalitis virus, and a Murray Valley encephalitis virus, Kunjin virus, and West Nile virus.

4. The nucleic acid of claim 1, wherein each of at least the first and second antigenic polypeptides comprises an envelope protein, a premembrane protein, or both an envelope protein and a premembrane protein.

5. The nucleic acid of claim 1, wherein at least the first and second

alphavirus.

6. The nucleic acid of claim 1, wherein at least the first and second antigenic polypeptides are different species or strains of a flavivirus or alphavirus.

7. A vector comprising the nucleic acid of claim 1.

8. The vector of claim 7, wherein the vector comprises an expression vector.

9. A host cell comprising the nucleic acid of claim 1.

10. A host cell comprising the vector of claim 7.

11. The host cell of claim 9, wherein the host cell is in vivo.

12. The host cell of claim 9, wherein the host cell expresses a polypeptide encoded by the nucleic acid.

13. A method of producing a recombinant **multivalent** antigenic polypeptide comprising culturing a host cell comprising the expression vector of claim 8 under conditions suitable for expression of the **multivalent** antigenic polypeptide.

14. The method of claim 13, further comprising isolating the **multivalent** antigenic polypeptide.

15. A composition comprising the nucleic acid of claim 1 and an excipient.

16. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces an immune response to the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any of the first and second antigenic polypeptides.

17. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces an immune response that is cross reactive against at least the first and second antigenic polypeptides and at least a third antigenic polypeptide of a flavivirus or alphavirus.

18. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces an immune response that is cross reactive against at least two different serotypes, strains, or species of a flavivirus or alphavirus.

19. The nucleic acid of claim 18, wherein the **multivalent** antigenic polypeptide induces an immune response that is cross reactive against at least three different serotypes, strains, or species of a flavivirus or alphavirus.

20. The nucleic acid of claim 19, wherein the **multivalent** antigenic polypeptide induces an immune response against a disease condition caused by one or more of at least three different serotypes, strains, or species.

21. A nucleic acid which encodes a recombinant **multivalent** antigenic polypeptide comprising multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, wherein the recombinant **multivalent** antigenic polypeptide is prepared by a method comprising: (1) recombining at least a first nucleic acid comprising a nucleotide sequence that encodes the first antigenic polypeptide and at least a second nucleic acid comprising a nucleotide sequence that encodes the second antigenic polypeptide, wherein at least the first and second nucleic acids differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids; and (2) screening the library of recombinant nucleic acids for at least one recombinant nucleic acid that encodes a recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first and second flaviviruses or alphaviruses that is greater than the immune response induced by the first antigenic polypeptide against the second flavivirus or alphavirus and the immune response induced by the second antigenic polypeptide against the first flavivirus or alphavirus.

22. The nucleic acid of claim 21, wherein the method further comprises: (3) recombining at least one recombinant nucleic acid with at least a third nucleic acid comprising a nucleotide sequence that encodes a third

third nucleic acid is the same or different from at least the first and second nucleic acids, to produce a further library of recombinant nucleic acids; (4) screening the further library of recombinant nucleic acids for at least one further recombinant nucleic acid that encodes a recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first, second and third flaviviruses or alphaviruses that is greater than the immune response induced by (i) the first antigenic polypeptide against the second or third flavivirus or alphavirus, (ii) the second antigenic polypeptide against the first or third flavivirus or alphavirus, and (iii) the third antigenic polypeptide against the first or second flavivirus or alphavirus; and (5) repeating (3) and (4), as necessary, for a further recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first, second and third flaviviruses or alphaviruses that is greater than the immune response induced by (i) the first antigenic polypeptide against the second or third flavivirus or alphavirus, (ii) the second antigenic polypeptide against the first or third flavivirus or alphavirus, and (iii) the third antigenic polypeptide against the first or second flavivirus or alphavirus.

23. The nucleic acid of claim 21, wherein at least the first and second antigenic polypeptides are different serotypes, species or strains of a flavivirus or alphavirus.

24. The nucleic acid of claim 22, wherein at least first, second, and third antigenic polypeptides are different serotypes, species or strains of a flavivirus or alphavirus.

25. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces an immune response against the first and second flaviviruses or alphaviruses that is greater than the immune response induced by the first antigenic polypeptide against the second flavivirus or alphavirus and the immune response induced by the second antigenic polypeptide against the first flavivirus or alphavirus.

26. The nucleic acid of claims 25, wherein the **multivalent** antigenic polypeptide induces an immune response against the fist and second flaviviruses or alphaviruses that is greater than the immune response induced by each of the first and second antigenic polypeptides against the fist flavivirus or alphavirus and the second flavivirus or alphavirus.

27. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against at least each of the first and second antigenic polypeptides.

28. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against at least the first flavivirus or alphavirus and the second flavivirus or alphavirus.

29. The nucleic acid of claim 2, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against at least each of the first, second, and third antigenic polypeptides.

30. The nucleic acid of claim 2, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against at least the first, second, and third flaviviruses or alphaviruses.

31. The nucleic acid of claim 2, wherein the **multivalent** antigenic polypeptide induces an immune response against at least the first, second, and third flaviviruses or alphaviruses.

32. The nucleic acid of claim 2, wherein the **multivalent** antigenic polypeptide induces an immune response against the first, second, and third antigenic polypeptides that is greater than the immune response induced by any one of the first, second, and third antigenic polypeptides against any other of the first, second, and third antigenic polypeptides.

33. A nucleic acid that encodes a **multivalent** antigenic polypeptide comprising multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against at least the first and second antigenic polypeptides.

34. The nucleic acid of claim 33, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against at

35. The nucleic acid of claim 33, wherein each of the first and second antigenic polypeptides comprises an envelope protein, a premembrane protein, or both an envelope protein and a premembrane protein.

36. The nucleic acid of claim 33, wherein the **multivalent** antigenic polypeptide is present as a component of a virus or a viral vector.

37. A composition comprising the nucleic acid of claim 33, and a carrier.

38. A vector comprising the nucleic acid of claim 33.

39. A host cell comprising the nucleic acid of claim 33.

40. A nucleic acid that encodes a **multivalent** antigenic polypeptide comprising multiple non-contiguous subsequences of a fast antigenic polypeptide of a **dengue**-1 virus, multiple non-contiguous subsequences of a second antigenic polypeptide of a **dengue**-2 virus, multiple non-contiguous subsequences of a third antigenic polypeptide of a **dengue**-3 virus, and multiple noncontiguous subsequences of a fourth antigenic polypeptide of a **dengue**-4 virus, each subsequence being positioned relative to its position in the first, second, third, or fourth antigenic polypeptide, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against the **dengue**-1 virus, **dengue**-2 virus, **dengue**-3 virus, and **dengue**-4 virus.

41. The nucleic acid of claim 40, wherein each of the first, second, third, and four antigenic polypeptides comprises an envelope protein, a premembrane protein, or both an envelope protein and a premembrane protein.

42. The nucleic acid of claim 40, wherein the **multivalent** antigenic polypeptide is present as a component of a virus or a viral vector.

43. A composition comprising the nucleic acid of claim 40 and a carrier.

44. A vector composing the nucleic acid of claim 40.

45. A host cell comprising the nucleic acid of claim 40.

46. The host cell of claim 10, wherein the host cell is in vivo.

47. The host cell of claim 10, wherein the host cell expresses a polypeptide encoded by the nucleic acid.

48. The host cell of claim 11, wherein the host cell expresses a polypeptide encoded by the nucleic acid.

49. The nucleic acid of claim 1, wherein the **multivalent** antigenic polypeptide induces an immune response against each of the at least first and second antigenic polypeptides that is greater than the immune response induced by any one of the at least first and second antigenic polypeptides against any other of the at least first and second antigenic polypeptides.

50. The nucleic acid of claim 33, wherein the **multivalent** antigenic polypeptide further comprises multiple non-contiguous subsequences of at least a third antigenic polypeptide of at least a third flavivirus or alphavirus.

51. The nucleic acid of claim 50, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against at least the first, second, and third antigenic polypeptides.

52. The nucleic acid of claim 50, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against at least the first, second, and third flaviviruses or alphaviruses.

53. The nucleic acid of claim 50, wherein the **multivalent** antigenic polypeptide induces production of neutralizing antibodies against each of the at least first, second, and third antigenic polypeptides or each of the at least first, second, and third flaviviruses or alphaviruses.

54. The nucleic acid of claim 2, wherein the **multivalent** antigenic polypeptide induces an immune response against the first, second, and third flaviviruses or alphaviruses that is greater than the immune response induced by any one of he first, second, and third antigenic polypeptides against any other of the first, second, and flaviviruses or alphaviruses.

2003:142838 Flavivirus and alphavirus recombinant antigen libraries.
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US 6569435 B1 20030527
APPLICATION: US 2000-724969 20001128 (9)
PRIORITY: US 1998-105509P 19981023 (60)
US 1998-74294P 19980211 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:

1. A recombinant antigen library comprising recombinant nucleic acids that encode antigenic polypeptides, wherein at least one recombinant nucleic acid encodes at least one recombinant **multivalent** antigenic polypeptide comprising multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, said at least one recombinant **multivalent** antigenic polypeptide inducing an immune response against each of the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any other of the first and second antigenic polypeptides.

2. A method of obtaining at least one polynucleotide that encodes at least one recombinant antigenic polypeptide that induces an immune response against different antigenic polypeptides, the method comprising: (1) recombining at least first and second nucleic acids, the first nucleic acid encoding at least a first antigenic polypeptide of a first flavivirus or alphavirus and the second nucleic acid encoding at least a second antigenic polypeptide of a second flavivirus or alphavivirus, wherein at least the first and second nucleic acids differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids that encode recombinant antigenic polypeptides; and (2) screening the library for at least one recombinant nucleic acid that encodes at least one recombinant antigenic polypeptide comprising multiple non-contiguous subsequences of at least the first antigenic polypeptide and multiple non-contiguous subsequences of at least the second antigenic polypeptide, each subsequence being positioned relative to its position in the respective antigenic polypeptide, which at least one recombinant antigenic polypeptide induces an immune response against each of the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any other of the first and second antigenic polypeptides.

3. The method of claim 2, wherein the method further comprises: (3) recombining at least one recombinant nucleic acid with a further nucleic acid, which is the same or different from at least the first and second nucleic acids, to produce a further library of recombinant nucleic acids; (4) screening the further library for at least one further recombinant nucleic acid that encodes at least one further recombinant antigenic polypeptide comprising multiple non-contiguous subsequences of at least the first antigenic polypeptide and multiple non-contiguous subsequences of at least the second antigenic polypeptide, each subsequence being positioned relative to its position in the respective antigenic polypeptide, which at least one further recombinant antigenic polypeptide induces an immune response against each of the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any other of the first and second antigenic polypeptides; and (5) repeating (3) and (4), as necessary, to screen for at least one further recombinant nucleic acid that encodes at least one further recombinant antigenic polypeptide that induces an immune response against each of the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any other of the first and second antigenic polypeptides.

4. The method of claim 2, wherein at least the first and second antigenic polypeptides are of different serotypes of a flavivirus or alphavirus.

5. The method of claim 2, wherein at least the first and second antigenic polypeptides are different species or strains of a flavivirus or alphavirus.

introducing into a test animal either: a) at least one recombinant nucleic acid of the library of recombinant nucleic acids, or b) at least one recombinant antigenic polypeptide of the recombinant antigenic polypeptides encoded by the library of recombinant nucleic acids; introducing a flavivirus or alphavirus into the test animal; and determining whether the test animal is resistant to challenge by the flavivirus or alphavirus.

7. The method of claim 6, wherein the flavivirus or alphavirus introduced into the test animal is of a different serotype than the first flavivirus or alphavirus or the second flavivirus or alphavirus.

8. The method of claim 6, wherein the library is subdivided into a plurality of pools, each of which pools is introduced into a test animal to screen for pools that include at least one recombinant nucleic acid that encodes at least one recombinant antigenic polypeptide that induces an immune response against each of the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any other of the first and second antigenic polypeptides.

9. The method of claim 8, wherein pools that include at least one recombinant nucleic acid are further subdivided into a plurality of subpools, each of which subpools is introduced into a test animal to screen for pools that include at least one recombinant nucleic acid that encodes at least one recombinant antigenic polypeptide that induces an immune response against each of the first and second antigenic polypeptides that is greater than the immune response induced by any one of the first and second antigenic polypeptides against any other of the first and second antigenic polypeptides.

10. The method of claim 2, wherein the screening is accomplished by: expressing each recombinant nucleic acid of the library of recombinant nucleic acids in a phage display expression vector such that at least one recombinant antigenic polypeptide encoded by a recombinant nucleic acid of the library is expressed as a fusion protein with a phage polypeptide that is displayed on a phage particle surface; contacting the phage with a first antibody that is specific for a first serotype of a flavivirus or alphavirus and selecting those phage that bind to the first antibody; and contacting those phage that bind to the first antibody with a second antibody that is specific for a second serotype of the flavivirus or alphavirus and selecting those phage that bind to the second antibody; wherein those phage that bind to the first antibody and the second antibody express a **multivalent** antigenic polypeptide.

11. The method of claim 10, wherein the screening further comprises contacting those phage that bind to the first and second antibodies with one or more additional antibodies, each of which is specific for an additional serotype of the flavivirus or alphavirus, and selecting those phage that bind to the respective additional antibodies.

12. The method of claim 10, wherein the phage display expression vector comprises a suppressible stop condon between the recombinant nucleic acid and nucleic acid encoding the phage polypeptide, whereby expression in a host cell which comprises a corresponding suppressor tRNA results in production of the fusion protein and expression in a host cell which lacks a corresponding suppressor tRNA results in production of the recombinant antigenic polypeptide not as a fusion protein.

13. The method of claim 2, wherein at least one recombinant antigenic polypeptide exhibits an enhanced expression level in a host cell compared to the expression level of the first and second antigenic polypeptides in the host cell, and the screening is accomplished by expression of each recombinant nucleic acid in the host cell and subjecting the host cells to flow cytometry-based cell sorting to obtain those host cells that display at least one recombinant antigenic polypeptide on the host cell surface.

14. The method of claim 2, wherein at least the first and second antigenic polypeptides are different serotypes, strains, or species of a flavivirus or alphavirus, and at least one recombinant nucleic acid encodes at least one recombinant antigenic polypeptide that induces production of neutralizing antibodies against each of the first and second antigenic polypeptides.

15. The method of claim 14, wherein at least one recombinant antigenic polypeptide induces production of neutralizing antibodies against at least one additional serotype, strain, or species of flavivirus or alphavirus.

16. The method of claim 4, wherein the screening is accomplished by: introducing into a test animal either: a) at least one of the

b) at least one of the recombinant antigenic polypeptides encoded by the library of recombinant nucleic acids; collecting serum from the test animal; and testing the serum for the presence of neutralizing antibodies against at least a first serotype of the flavivirus or alphavirus.

17. The method of claim 16, further comprising testing the serum for the presence of neutralizing antibodies against at least a second serotype of the flavivirus or alphavivirus that is different from the first serotype.

18. The method of claim 17, wherein each of the first and second antigenic polypeptides is a **dengue** virus antigen.

19. The method of claim 18, further comprising testing the serum for the presence of neutralizing antibodies against at least four different serotypes of **dengue** virus.

20. The method of claim 2, wherein (2) comprises screening the library for at least one recombinant nucleic acid that encodes at least one recombinant antigenic polypeptide that induces an immune response against each of the first and second antigenic polypeptides that is greater that the immune response induced by any one of the first and second antigenic polypeptides against any of the first and second antigenic polypeptides.

21. The method of claim 2, wherein at least the first and second antigenic polypeptides are of a virus selected from the group consisting of an equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus complex, a virus of the tick-borne encephalitis virus complex, a **Dengue** virus, a yellow fever virus, a St. Louis encephalitis virus, and a Murray Valley encephalitis virus.

22. The method of claim 20, wherein the flavivirus or alphavirus introduced into the test animal is of the same serotype as the first or second flavivirus or alphavirus.

23. The recombinant antigen library of claim 1, wherein at least one recombinant nucleic acid in the library encodes at least one recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first and second antigenic polypeptides that is greater than the immune induced by any one of the first and second antigenic polypeptides against any of the first and second antigenic polypeptides.

24. The recombinant antigen library of claim 23, wherein at least one recombinant nucleic acid in the library encodes at least one recombinant **multivalent** antigenic polypeptide that induces neutralizing antibodies against each of the first and second antigenic polypeptides.

25. The recombinant antigen library of claim 1, wherein at least one recombinant nucleic acid in the library encodes at least one recombinant **multivalent** antigenic polypeptide that induces neutralizing antibodies each of the at least first and second antigenic polypeptides.

26. The recombinant antigen library of claim 1, wherein each of the first and second antigenic polypeptides comprises an envelope protein, a premembrane protein, or both an envelope protein and premembrane protein.

27. The recombinant antigen library of claim 1, wherein at least the first and second antigenic polypeptides are of a virus selected from the group consisting of an equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus complex, a virus of the tick-bone encephalitis virus complex, a **Dengue** virus, a yellow fever virus, a St. Louis encephalitis virus, and a Murray Valley encephalitis virus.

28. The recombinant antigen library of claim 1, wherein at least one recombinant **multivalent** antigenic polypeptide induces an immune response that is cross reactive against at least the first and second antigenic polypeptides or first and second flaviviruses or alphaviruses and against at least a third antigenic polypeptide of a flavivirus or alphavirus or against a third flavivirus or alphavirus.

29. The recombinant antigen library of claim 1, wherein at least one recombinant **multivalent** antigenic polypeptide comprises multiple non-contiguous subsequences of at least a third antigenic polypeptide of at least a third flavivirus or alphavirus.

30. The recombinant antigen library of claim 29, wherein at least one recombinant **multivalent** antigenic polypeptide induces an immune

polypeptides that is greater than the immune response induced by any one of the first, second, and third antigenic polypeptides against any other of the first, second, and third antigenic polypeptides.

31. The recombinant antigen library of claim 30, wherein at least one recombinant **multivalent** antigenic polypeptide induces an immune response against each of the first, second, and third antigenic polypeptides that is greater than the immune response induced by any one of the first, second, and third antigenic polypeptides against any of the first, second, and third antigenic polypeptides.

32. The recombinant antigen library of claim 30, wherein the first, second, and third antigenic polypeptides are different serotypes, strains, or species of a flavivirus or alphavirus.

33. The recombinant antigen library of claim 1, wherein at least one recombinant nucleic acid in the library encodes at least one recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first and second flaviviruses or alphaviruses that is greater than the immune response induced by the first antigenic polypeptide against the second flavivirus or alphavirus and the immune response induced by the second antigenic polypeptide against the first flavivirus or alphavirus.

34. The recombinant antigen library of claim 33, wherein at least one recombinant nucleic acid in the library encodes at least one recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the first and second flaviviruses or alphaviruses that is greater than the immune response induced by the first or second antigenic polypeptide against any of the first or second flavivirus or alphavirus.

35. The recombinant antigen library of claim 1, wherein at least one recombinant nucleic acid in the library encodes at least one recombinant **multivalent** antigenic polypeptide that induces neutralizing antibodies against one or more of the first and second flaviviruses or alphaviruses.

36. The recombinant antigen library of claim 35, wherein at least one recombinant nucleic acid in the library encodes at least one recombinant **multivalent** antigenic polypeptide that induces neutralizing antibodies against each of the at least first and second flaviviruses or alphaviruses.

37. The recombinant antigen library of claim 1, wherein at least the first and second flaviviruses or alphaviruses are of different species, serotypes, or strains, and at least one recombinant **multivalent** antigenic polypeptide induces neutralizing antibodies against one or more of the first and second antigenic polypeptides or one or more of the first and second flaviviruses or alphaviruses.

38. The recombinant antigen library of claim 1, wherein at least one recombinant **multivalent** antigenic polypeptide induces a neutralizing antibody response against at least three different serotypes, species or strains of flavivirus or alphavirus.

39. The recombinant antigen library of claim 38, wherein at least one recombinant **multivalent** antigenic polypeptide induces a neutralizing antibody responses against at least four different viral serotypes, species or strains of flavivirus or alphavirus.

40. The recombinant antigen library of claim 29, wherein at least one recombinant **multivalent** antigenic polypeptide further comprises multiple non-contiguous subsequences of at least a fourth antigenic polypeptide of at least a fourth flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide.

41. The recombinant antigen library of claim 40, wherein the first, second, third, and fourth flavivirus or alphaviruses are of different serotypes.

42. The recombinant antigen library of claim 40, wherein the first, second, third, and fourth flaviviruses or alphaviruses are **dengue**-1 virus, **dengue**-2 virus, **dengue**-3 virus, and **dengue**-4 virus, respectively.

43. The recombinant antigen library of claim 42, wherein at least one recombinant **multivalent** antigenic polypeptide induces an immune response against each of a **dengue**-1 virus, **dengue**-2 virus, **dengue**-3 virus, and **dengue**-4 virus that is greater than: (i) the immune response induced by the first antigenic polypeptide against a **dengue**-2 virus, **dengue**-3 virus, or **dengue**-4 virus; (ii) the immune

dengue-1 virus, **dengue**-3 virus, or **dengue**-4 virus; (iii) the immune response induced by the third antigenic polypeptide against a **dengue**-1 virus, **dengue**-2 virus, or **dengue**-4 virus; and (iv) the immune response induced by the fourth antigenic polypeptide against a **dengue**-1 virus, **dengue**-2 virus, or **dengue**-3 virus.

44. A recombinant antigen library comprising recombinant nucleic acids that encode recombinant antigenic polypeptides, wherein the library comprises at least one recombinant nucleic acid that encodes at least one recombinant **multivalent** antigenic polypeptide comprising multiple non-contiguous subsequences of at least a first antigenic polypeptide of at least a first flavivirus or alphavirus, and multiple non-contiguous subsequences of at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, each subsequence being positioned relative to its position in the respective antigenic polypeptide, said at least one recombinant **multivalent** antigenic polypeptide inducing production of neutralizing antibodies against at least the first and second antigenic polypeptides or the first and second flaviviruses or alphaviruses.

45. The recombinant antigen library of claim 1, wherein at least one recombinant nucleic acid in the library encodes at least one recombinant **multivalent** antigenic polypeptide that induces an immune response against each of the at least first and second antigenic polypeptides that is greater than the immune response induced by a combination of all of the at least first and second antigenic polypeptides against any of the at least first and second antigenic polypeptides.

46. The recombinant antigen library of claim 44, wherein at least one recombinant **multivalent** antigenic polypeptide further comprises multiple non-contiguous subsequences of at least a third antigenic polypeptide of at least a third flavivirus or alphavirus.

47. The recombinant antigen library of claim 46, wherein at least one recombinant **multivalent** antigenic polypeptide induces neutralizing antibodies against the first, second, and third antigenic polypeptides or against the first, second, and third flaviviuses or alphaviruses.

48. The recombinant antigen library of claim 47, at least one recombinant **multivalent** antigenic polypeptide further comprises multiple non-contiguous subsequences of a fourth antigenic polypeptide of a fourth flavivirus or alphavirus.

49. The recombinant antigen library of claim 48, wherein at least one recombinant **multivalent** antigenic polypeptide induces neutralizing antibodies against the first, second, third, and fourth polypeptides or against the first, second, third, or fourth flaviviruses or alphaviruses.

50. The recombinant antigen library of claim 46, wherein each of the first, second, and third antigenic polypeptides comprises an envelope protein, a premembrane protein, or an envelope protein and premembrane protein.

51. The recombinant antigen library of claim 1, wherein the library is obtained by recombining at least first and second nucleic acids, the at least first nucleic acid encoding at least a first antigenic polypeptide of at least a first flavivirus or alphavirus and the at least second nucleic acid encoding at least a second antigenic polypeptide of at least a second flavivirus or alphavirus, wherein the at least first and second nucleic acids differ from each other in two or more nucleotide, to produce a library of recombinant nucleic acids.

L32 ANSWER 5 OF 14 USPATFULL on STN
2002:344432 ANTIGEN LIBRARY IMMUNIZATION.
PUNNONEN, JUHA, PALO ALTO, CA, UNITED STATES
BASS, STEVEN H., HILLSBOROUGH, CA, UNITED STATES
WHALEN, ROBERT GERALD, PARIS, FRANCE
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US 2002198162 A1 20021226
APPLICATION: US 1999-247890 A1 19990210 (9)
PRIORITY: US 1998-74294P 19980211 (60)
US 1998-105509P 19981023 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An recombinant **multivalent** antigenic polypeptide that comprises a first antigenic determinant of a first polypeptide and at least a second antigenic determinant from a second polypeptide.

polypeptide comprises at least a third antigenic determinant from a third polypeptide.

3. The **multivalent** antigenic polypeptide of claim 1, wherein the first and second polypeptides are selected from the group consisting of cancer antigens, antigens associated with autoimmunity disorders, antigens associated with inflammatory conditions, antigens associated with allergic reactions, and antigens from infectious agents.

4. The **multivalent** antigenic polypeptide of claim 3, wherein the antigens are from a virus, a parasite, or a bacteria.

5. The **multivalent** antigenic polypeptide of claim 4, wherein the antigens are from a virus selected from the group consisting of a Venezuelan equine encephalitis virus or a related alphavirus, a virus of the Japanese encephalitis virus complex, a virus of the tick-borne encephalitis virus complex, a **Dengue** virus, a Hanta virus, an HIV, a hepatitis B virus, a hepatitis C virus, and a Herpes simplex virus.

6. The **multivalent** antigenic polypeptide of claim 5, wherein the antigens are envelope proteins.

7. The **multivalent** antigenic polypeptide of claim 4, wherein the antigens are from a bacteria and are selected from the group consisting of a Yersinia V antigen, a Staphylococcus aureus enterotoxin, a Streptococcus pyogenes enterotoxin, a Vibrio cholera toxin, an enterotoxigenic Escherichia coli heat labile enterotoxin, a OspA and a OspC polypeptide from a Borrelia species, an Antigen 85 polypeptide from a Mycobacterium species, a VacA and a CagA polypeptide from Helicobacter pylori, and an MSP antigen from Plasmodium falciparum.

8. The **multivalent** antigenic polypeptide of claim 1, wherein the **multivalent** antigenic polypeptide exhibits reduced affinity to IgE from a mammal compared to the first or second polypeptides.

9. The **multivalent** antigenic polypeptide of claim 1, wherein the first antigenic determinant and the second antigenic determinant are from different serotypes of a pathogenic organism.

10. The **multivalent** antigenic polypeptide of claim 1, wherein the first antigenic determinant and the second antigenic determinant are from different species of pathogenic organism.

11. The **multivalent** antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are allergens.

12. The **multivalent** antigenic polypeptide of claim 11, wherein the allergens are dust mite allergens, grass pollen allergens, birch pollen allergens, ragweed pollen allergens, hazel pollen allergens, cockroach allergens, rice allergens, olive tree pollen allergens, fungal allergens, mustard allergens, and bee venom.

13. The **multivalent** antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are associated with an inflammatory or autoimmune disease.

14. The **multivalent** antigenic polypeptide of claim 13, wherein the first polypeptide and the second polypeptide are autoantigens associated with a disease selected from the group consisting of multiple sclerosis, scleroderma, systemic sclerosis, systemic lupus erythematosus, hepatic autoimmune disorder, skin autoimmune disorder, insulin-dependent diabetes mellitus, thyroid autoimmune disorder, and rheumatoid arthritis.

15. The **multivalent** antigenic polypeptide of claim 1, wherein the first polypeptide and the second polypeptide are cancer antigens or sperm antigens.

16. A recombinant antigen library comprising recombinant nucleic acids that encode antigenic polypeptides, wherein the library is obtained by recombining at least first and second forms of a nucleic acid which comprises a polynucleotide sequence that encodes a disease-associated antigenic polypeptide, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids.

17. The recombinant antigen library of claim 16, wherein the first and second polypeptides are toxins.

18. A method of obtaining a polynucleotide that encodes a recombinant antigen having improved ability to induce an immune response to a disease condition, the method comprising: (1) recombining at least

polynucleotide sequence that encodes an antigenic polypeptide that is associated with the disease condition, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids; and (2) screening the library to identify at least one optimized recombinant nucleic acid that encodes an optimized recombinant antigenic polypeptide that has improved ability to induce an immune response to the disease condition.

19. The method of claim 18, wherein the method further comprises: (3) recombining at least one optimized recombinant nucleic acid with a further form of the nucleic acid, which is the same or different from the first and second forms, to produce a further library of recombinant nucleic acids; (4) screening the further library to identify at least one further optimized recombinant nucleic acid that encodes a polypeptide that has improved ability to induce an immune response to the disease condition; and (5) repeating (3) and (4), as necessary, until the further optimized recombinant nucleic acid encodes a polypeptide that has improved ability to induce an immune response to the disease condition.

20. The method of claim 18, wherein the disease-associated polypeptides are selected from the group consisting of cancer antigens, antigens associated with autoimmunity disorders, antigens associated with inflammatory conditions, antigens associated with allergic reactions, and antigens associated with infectious agents.

21. The method of claim 18, wherein the disease condition is an infectious disease and the first and second forms of the nucleic acid each encode an antigen of a different serotype of a pathogenic agent.

22. The method of claim 21, wherein the first and second forms of the nucleic acid are each from a different species of pathogen.

23. The method of claim 21, wherein the screening is accomplished by: introducing into a test animal either: a) the library of recombinant nucleic acids, or b) recombinant polypeptides encoded by the library of recombinant nucleic acids; introducing the pathogenic agent into the test animal; and determining whether the test animal is resistant to challenge by the pathogenic agent.

24. The method of claim 23, wherein the pathogenic agent introduced into the test animal is of a different serotype than that used as a source of the first and second forms of the nucleic acid.

25. The method of claim 23, wherein the library is subdivided into a plurality of pools, each of which pools is introduced into a test animal to identify those pools that include an optimized recombinant nucleic acid that encodes a polypeptide which has improved ability to induce an immune response to the pathogenic agent.

26. The method of claim 25, wherein the pools that include an optimized recombinant nucleic acid are further subdivided into a plurality of subpools, each of which subpools is introduced into a test animal to identify those pools that include an optimized recombinant nucleic acid that encodes a polypeptide which has improved ability to induce an immune response to the pathogenic agent.

27. The method of claim 18, wherein the optimized recombinant nucleic acid encodes a **multivalent** antigenic polypeptide and the screening is accomplished by: expressing the library of recombinant nucleic acids in a phage display expression vector such that the recombinant antigen is expressed as a fusion protein with a phage polypeptide that is displayed on a phage particle surface; contacting the phage with a first antibody that is specific for a first serotype of the pathogenic agent and selecting those phage that bind to the first antibody; contacting those phage that bind to the first antibody with a second antibody that is specific for a second serotype of the pathogenic agent and selecting those phage that bind to the second antibody; wherein those phage that bind to the first antibody and the second antibody express a **multivalent** antigenic polypeptide.

28. The method of claim 27, wherein the screening further comprises contacting those phage that bind to the first and second antibodies with one or more additional antibodies, each of which is specific for an additional serotype of the pathogenic agent, and selecting those phage that bind to the respective additional antibodies.

29. The method of claim 27, wherein the phage display expression vector comprises a suppressible stop codon between the recombinant nucleic acid and the phage polypeptide, whereby expression in a host cell which comprises a corresponding suppressor tRNA results in production of the fusion protein and expression in a host cell which lacks a corresponding

a fusion protein.

30. The method of claim 18, wherein the optimized recombinant antigen exhibits an enhanced expression level in a host cell and the screening is accomplished by expression of each recombinant nucleic acid in the host cell and subjecting the host cells to flow cytometry-based cell sorting to obtain those host cells that display the recombinant antigen on the host cell surface.

31. The method of claim 18, wherein the improved property is selected from the group consisting of: improved immunogenicity; enhanced cross-reactivity against different forms of the disease-associated antigenic polypeptide; reduced toxicity; improved adjuvant activity in vivo; and improved production of the immunogenic polypeptide.

32. The method of claim 31, wherein the improved property is enhanced cross-reactivity against different forms of the disease-associated polypeptide and the first and second forms of the nucleic acid are from a first and a second form of the disease-associated polypeptide.

33. The method of claim 32, wherein the first and second forms of the disease-associated polypeptide are obtained from at least a first and second species of a pathogenic agent and the optimized recombinant nucleic acid encodes a recombinant polypeptide that induces a protective response against both species of the pathogenic agent.

34. The method of claim 33, wherein the recombinant polypeptide induces a protective response against at least one additional species of the pathogenic agent.

35. The method of claim 33, wherein the pathogenic agent is a toxin.

36. The method of claim 33, wherein the pathogenic agent is a virus or a cell.

37. The method of claim 33, wherein the disease-associated polypeptide is a Yersinia V-antigen.

38. The method of claim 37, wherein the at least first and second forms of a nucleic acid are obtained from at least a first and second species of Yersinia.

39. The method of claim 38, wherein the Yersinia species are selected from the group consisting of Y. pestis, Y. enterocolitica, and Y. pseudotuberculosis.

40. The method of claim 33, wherein the pathogenic agent is a bacterial toxin.

41. The method of claim 18, wherein the disease condition is cancer and the screening step involves introducing the optimized recombinant nucleic acids into a genetic **vaccine** vector and testing library members for ability to inhibit proliferation of cancer cells or inducing death of cancer cells.

42. The method of claim 41, wherein the optimized recombinant nucleic acid comprises a nucleotide sequence that encodes a tumor specific antigen.

43. The method of claim 41, wherein the optimized recombinant nucleic acid comprises a nucleotide sequence that encodes a molecule which is capable of inhibiting proliferation of cancer cells.

44. The method of claim 18, wherein the disease condition is an inflammatory response which has an unknown or no antigen specificity and the screening step involves one or more of the following: a) determining the ability of the genetic **vaccine** vector to induce cytokine production by PBMC, synovial fluid cells, purified T cells, monocytes/macrophages, dendritic cells, or T cell clones; b) determining the ability of the genetic **vaccine** vector to induce T cell activation or proliferation; and c) determining the ability of the genetic **vaccine** vector to induce T cell differentiation to T_{H1} or T_{H2} cells.

45. The method of claim 18, wherein the disease condition is an autoimmune response.

46. The method of claim 45, wherein the optimized recombinant antigenic polypeptide shifts the immune response from a $T_{H1\text{-mediated}}$ response to a $T_{H2\text{-mediated}}$ response.

47. The method of claim 18, wherein the disease condition is an allergic

48. The method of claim 47, wherein the optimized recombinant antigenic polypeptide shifts the immune response from a $T_{H2\text{-mediated}}$ response to a $T_{H1\text{-mediated}}$ response.

49. The method of claim 47, wherein the optimized recombinant antigenic polypeptide induces an immune response characterized by predominant IgG and IgM expression and reduced IgE expression.

50. The method of claim 47, wherein the optimized recombinant antigenic polypeptide is not recognized by pre-existing IgE molecules present in sera of atopic mammals.

51. The method of claim 50, wherein the optimized recombinant antigenic polypeptide retains T cell epitopes that are involved in modulating a T cell response.

52. A method of obtaining a recombinant viral vector which has an enhanced ability to induce an antiviral response in a cell, the method comprising the steps of: (1) recombining at least first and second forms of a nucleic acid which comprise a viral vector, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant viral vectors; (2) transfecting the library of recombinant viral vectors into a population of mammalian cells; (3) staining the cells for the presence of Mx protein; and (4) isolating recombinant viral vectors from cells which stain positive for Mx protein, wherein recombinant viral vectors from positive staining cells exhibit enhanced ability to induce an antiviral response.

53. The method of claim 52, wherein the viral vector comprises an influenza viral genomic nucleic acid.

L32 ANSWER 6 OF 14 USPATFULL on STN
2001:102384 Inactivated **dengue** virus **vaccine**.
Putnak, J. Robert, Silver Spring, MD, United States
Eckels, Kenneth, Rockville, MD, United States
Dubois, Doris R., Wheaton, MD, United States
The United States of America as represented by the Secretary of the Army, Washington, DC, United States (U.S. government)
US 6254873 B1 20010703
APPLICATION: US 1995-423338 19950417 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A **vaccine** comprising a purified, inactivated **dengue** virus effective for inducing a protective immune response in primates against **dengue** virus.

2. The purified, inactivated **dengue** virus **vaccine** of claim 1 comprising a first inactivated **dengue** virus, wherein said inactivated **dengue** virus is selected from the group consisting of **dengue** virus type 1, **dengue** virus type 2, **dengue** virus type 3, and **dengue** virus type 4.

3. The purified, inactivated **dengue** virus **vaccine** of claim 2, wherein said **dengue** virus **vaccine** further comprises of one or more purified inactivated **dengue** virus serotype(s) different from the first inactivated **dengue** virus, wherein said purified, inactivated **dengue** virus is selected from the group consisting of **dengue** virus type 1, **dengue** virus type 2, **dengue** virus type 3 and **dengue** virus type 4.

4. A method for producing antibodies against **dengue** virus comprising administering to an individual a purified, inactivated **dengue** virus **vaccine** according to claim 1 in an amount sufficient to produce said antibodies wherein said **vaccine** is chosen from the group consisting of **dengue** virus type 1 **vaccine**, **dengue** virus type 2 **vaccine**, **dengue** virus type 3 **vaccine** and **dengue** virus type 4 **vaccine**.

5. A method for producing the purified, inactivated **dengue** virus **vaccine** of claim 1 comprising the steps of: (i) propagating **dengue** virus in a cell culture; (ii) harvesting said virus from said cell culture; (iii) concentrating said virus; (iv) purifying said virus such that it is essentially free of cell culture proteins and DNA; (v) inactivating said virus; and (vi) adding a suitable adjuvant in a pharmaceutically acceptable amount.

6. A method for producing a purified, inactivated **dengue** virus **vaccine** according to claim 5, further comprising the step of removing serum protein supplements prior to harvesting said virus according to step (ii).

said inactivated **dengue** virus is chemically inactivated.

8. The purified, inactivated **dengue** virus **vaccine** of claim 7 comprising a first inactivated **dengue** virus, wherein said first inactivated **dengue** virus is selected from the group consisting of **dengue** virus type 1, **dengue** virus type 2, **dengue** virus type 3, and **dengue** virus type 4.

9. The purified, inactivated **dengue** virus **vaccine** of claim 8, wherein said **dengue** virus **vaccine** further comprises an additional one or more purified inactivated **dengue** virus serotype(s) different from said first inactivated **dengue** virus, wherein said additional inactivated **dengue** virus is selected from the group consisting of **dengue** virus type 1, **dengue** virus type 2, **dengue** virus type 3, and **dengue** virus type 4.

10. The purified, inactivated **dengue** virus **vaccine** according to claim 1 wherein the specific activity of said **vaccine** is at least about 1×10^8 plaque forming units per milligram of total protein.

11. The purified inactivated **dengue** virus **vaccine** of claim 1, wherein said **dengue** virus **vaccine** is **dengue** 1 virus **vaccine** produced from deposited **dengue** 1 virus having ATCC accession no. VR-2649.

12. The purified inactivated **dengue** virus **vaccine** of claim 1, wherein said **dengue** virus **vaccine** is **dengue** 2 virus **vaccine** produced from deposited **dengue** 2 virus having ATCC accession no. VR-2650.

13. The purified inactivated **dengue** virus **vaccine** of claim 1, wherein said **dengue** virus **vaccine** is **dengue** 3 virus **vaccine** produced from deposited **dengue** 3 virus having ATCC accession no. VR-2654.

14. The purified inactivated **dengue** virus **vaccine** of claim 1, wherein said **dengue** virus **vaccine** is **dengue** 4 virus **vaccine** produced from deposited **dengue** 4 virus having ATCC accession no. VR-2651.

15. A method for inducing an immune response in an individual against **dengue** virus comprising administering to an individual a purified, inactivated **dengue** virus **vaccine** according to claim 1 in an amount sufficient to produce said immune response wherein said **vaccine** is chosen from the group consisting essentially of **dengue** virus type 1 **vaccine, dengue** virus type 2 **vaccine, dengue** virus type 3 **vaccine** and **dengue** virus type 4 **vaccine** or any combination thereof.

16. A **dengue** 2 virus **vaccine** produced by inactivating a **dengue** 2 virus deposited at ATCC under accession no. VR2650.

17. A **multivalent vaccine** comprising purified inactivated **dengue** virus propagated in vertebrate tissue culture cells and effective for inducing a protective immune response in primates against **dengue** virus wherein said virus is chosen from the group consisting of purified inactivated **dengue** virus type 1, purified inactivated **dengue** virus type 2, purified inactivated **dengue** virus type 3, and purified inactivated **dengue** virus type 4.

18. An isolated **dengue** type 1 virus having ATCC accession number VR-2649.

19. An isolated **dengue** type 1 virus **vaccine** produced by inactivating a **dengue** 1 virus of claim 18.

20. An isolated cell infected with the **dengue** type 1 virus of claim 18.

21. An isolated **dengue** type 2 virus having ATCC accession number VR-2650.

22. An isolated cell infected with the **dengue** type 2 virus of claim 21.

23. An isolated **dengue** type 3 virus having ATCC accession number VR-2654.

24. An isolated **dengue** type 3 virus **vaccine** produced by inactivating a **dengue** 3 virus of claim 23.

25. An isolated cell infected with the **dengue** type 3 virus of claim 23.

26. An isolated **dengue** type 4 virus having ATCC accession number

27. An isolated **dengue** type 4 virus **vaccine** produced by inactivating a **dengue** 4 virus of claim 26.

28. An isolated cell infected with the **dengue** type 4 virus of claim 26.

L32 ANSWER 7 OF 14 USPATFULL on STN
2001:33065 Method and compositions for isolation, diagnosis and treatment of polyanion-binding microorganisms.
Marks, Rory M., Ann Arbor, MI, United States
Chen, Yaping, San Diego, CA, United States
Maguire, Terence, Waverly Dunedin, New Zealand
Linhardt, Robert J., Iowa City, IA, United States
The Regents of the University of Michigan, Ann Arbor, MI, United States (U.S. corporation)
US 6197568 B1 20010306
APPLICATION: US 1998-123770 19980728 (9)
PRIORITY: US 1997-53828P 19970729 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for detecting a polyanion-binding microorganism in a biological sample, said method comprising: a) contacting the biological sample with an immobilized polyanion to which the microorganism from said biological sample adheres to form a polyanion-microorganism complex while the remainder of the biological sample is non-adherent to said polyanion; b) analyzing the complex to determine whether the microorganism is present; and c) releasing intact microorganism from said complex.

2. The method of claim 1, wherein the microorganism is a virus.

3. The method of claim 2, wherein the virus is a hemorrhagic fever virus.

4. The method of claim 2, wherein the virus is Hepatitis C.

5. The method of claim 2, wherein the virus is a flavivirus.

6. The method of claim 5, wherein the virus is a **dengue** virus.

7. The method of claim 6, wherein the **dengue** virus binds the polyanion through a polyanion binding motif having the sequence of SEQ ID NO:1.

8. The method of claim 6, wherein the **dengue** virus binds the polyanion through a polyanion binding motif having the sequence of SEQ ID NO:2.

9. The method of claim 1, wherein the polyanion is selected from the group consisting of heparin, highly sulfated heparan sulfate, a synthetic polyanion, and a **dengue** virus envelope protein receptor derived from Vero cell glycosaminoglycan (GAG) or endothelial mucin or analogues thereof.

10. The method of claim 1, wherein said immobilized polyanion is a sulfated heparin-derived decasaccharide.

L32 ANSWER 8 OF 14 USPATFULL on STN
2000:1737 Method of screening for attenuating viruses.
Barrett, Alan, Galveston, TX, United States
Ni, Haolin, Galveston, TX, United States
Ryman, Kate, Carrboro, NC, United States
Research Development Foundation, Carson City, NV, United States (U.S. corporation)
US 6010894 20000104
APPLICATION: US 1997-874272 19970613 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method of selecting virus **vaccine** candidates by selecting virus variants that do not bind to brain membrane receptor preparations, comprising the steps of: (a) preparing a brain membrane receptor preparation; (b) mixing an amount of a virus of interest with an amount of said membrane receptor preparation containing excess membrane receptors to form a virus-membrane receptor preparation suspension; (c) centrifuging said suspension to form a supernatant; (d) determining residual virus infectivity in said supernatant; and (e) isolating individual membrane receptor preparation binding-resistant virus variants which are useful as virus **vaccine** candidates.

2. The method of claim 1, wherein said brain membranes are selected from

and human brain membranes.

3. The method of claim 1, wherein said brain membrane receptor preparation has a protein concentration of about 20-40 mg wet brain per ml.

4. The method of claim 1, wherein said residual virus infectivity in said supernatant is determined by infecting a cell monolayer and counting plaques produced.

5. The method of claim 1, wherein said the individual membrane receptor preparation binding-resistant variant plaques and the viruses are amplified.

6. The method of claim 1, wherein said variants generated are incubated with fresh brain membrane receptor preparations and a lack of binding of the variants to fresh brain membrane receptor preparations confirm that the variants are true variants.

7. The method of claim 1, wherein said variants are **attenuated** for neuroinvasiveness and neurovirulence.

8. The method of claim 1, wherein said virus is selected from the group consisting of yellow fever, Japanese encephalitis, **dengue**-4 and langat.

L32 ANSWER 9 OF 14 USPATFULL on STN
1999:48203 Non-antibiotic system for selection of recombinant mycobacteria.
Barrett, Alan D. T., Galveston, TX, United States
Niesel, David, League City, TX, United States
Robb, Christopher, Galveston, TX, United States
Ni, Haolin, Galveston, TX, United States
The Board of Trustees of the University of Texas System, Austin, TX, United States (U.S. corporation)
US 5895756 19990420
APPLICATION: US 1997-840101 19970411 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A Mycobacterium-E. coli shuttle vector containing a non-mycobacterial, non-e. coli heterologous protein antigen sequence as a tribrid fusion with the M. leprai 18kDa antigen sequence and a phoA protein antigen sequence.

2. The shuttle vector of claim 1, wherein said vector is pCR7.

3. The shuttle vector of claim 1, wherein said phoA sequence is a E. coli phoA.

4. The shuttle vector of claim 1, wherein said heterologous protein antigen is **dengue** virus-4 E protein.

5. The shuttle vector of claim 1, wherein said vector is useful to select for recombinant mycobacteria using calorimetric detection.

6. The shuttle vector of claim 1, wherein said vector is useful to select for recombinant mycobacteria using an alkaline phosphatase based detection technique.

L32 ANSWER 10 OF 14 USPATFULL on STN
1998:68530 Trova fowl pox virus recombinants comprising heterologous inserts.
Paoletti, Enzo, Delmar, NY, United States
Perkus, Marion E., Altamont, NY, United States
Taylor, Jill, Albany, NY, United States
Tartaglia, James, Schenectady, NY, United States
Norton, Elizabeth K., Latham, NY, United States
Riviere, Michel, Ecully, France
de Taisne, Charles, Lyons, France
Limbach, Keith J., Troy, NY, United States
Johnson, Gerard P., Waterford, NY, United States
Pincus, Steven E., East Greenbush, NY, United States
Cox, William I., Troy, NY, United States
Audonnet, Jean-Christophe Francis, Albany, NY, United States
Gettig, Russell Robert, Averill Park, NY, United States
Virogenetics Corporation, Troy, NY, United States (U.S. corporation)
US 5766599 19980616
APPLICATION: US 1995-458101 19950601 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An **attenuated** virus having all the identifying characteristics of:

2. A virus which is TROVAC.

3. A vector which comprises the virus of claim 1.

4. A vector which comprises the virus of claim 2.

5. A virus as claimed in claim 2 further comprising exogenous DNA from a non-poxvirus source in a nonessential region of the virus genome.

6. A virus as claimed in claim 5 wherein the exogenous DNA is selected from the group consisting of rabies virus, Hepatitis B virus, Japanese encephalitis virus, yellow fever virus, **Dengue** virus, measles virus, pseudorabies virus, Epstein-Barr virus, herpes simplex virus, human immunodeficiency virus, simian immunodeficiency virus, equine herpes virus, bovine herpes virus, bovine viral diarrhea virus, human cytomegalovirus, canine parvovirus, equine influenza virus, feline leukemia virus, feline herpes virus, Hantaan virus, C. tetani, avian influenza virus, mumps virus and Newcastle Disease virus.

7. A virus as claimed in claim 6 wherein the non-poxvirus source is avian influenza virus and the fowlpox virus is vFP89, vFP92, vFP100 or vFP122.

8. A virus as claimed in claim 6 wherein the virus is a fowlpox virus, the non-poxvirus source is human immunodeficiency virus and the fowlpox virus is vFP62, vFP63 or vFP174.

9. A virus as claimed in claim 6 wherein the non-poxvirus source is Newcastle Disease virus and the fowlpox virus is vFP96.

10. A virus as claimed in claim 6 which is a human immunodeficiency virus recombinant fowlpox virus which is vFP62 or vFP63.

11. A virus as claimed in claim 1 further comprising exogenous DNA from a non-poxvirus source in a nonessential region of the virus genome.

12. An immunological composition for inducing an immunological response in a host animal inoculated with said composition, said composition comprising the virus of any one of claims 1, 2 or 10 or 11, or, a vector as claimed in claim 3 or 4, and a carrier.

13. The immunological composition of claim 12 containing the virus or vector in an amount sufficient to induce a protective immunological response such that the immunological composition is a **vaccine**.

14. A method of expressing a gene product in a cell cultured in vitro comprising introducing into the cell a virus as claimed in any one of claims 1, 2 or 10 or 11, or, a vector as claimed in claim 3 or 4, transforming cell with the expression vector, cultivating the transformed cell under conditions which allow expression of the gene product, and further purifying the product.

L32 ANSWER 11 OF 14 USPATFULL on STN
1998:57530 Alvac canarypox virus recombinants comprising heterlogous inserts.
Paoletti, Enzo, Delmar, NY, United States
Perkus, Marion E., Altamont, NY, United States
Taylor, Jill, Albany, NY, United States
Tartaglia, James, Schenectady, NY, United States
Norton, Elizabeth K., Latham, NY, United States
Riviere, Michel, Ecully, France
de Taisne, Charles, Lyons, France
Limbach, Keith J., Troy, NY, United States
Johnson, Gerard P., Waterford, NY, United States
Pincus, Steven E., East Greenbush, NY, United States
Cox, William I., Troy, NY, United States
Audonnet, Jean-Christophe Francis, Albany, NY, United States
Gettig, Russell Robert, Averill Park, NY, United States
Virogenetics Corporation, Troy, NY, United States (U.S. corporation)
US 5756103 19980526
APPLICATION: US 1995-457007 19950601 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. An **attenuated** virus having all the identifying characteristics of: an ALVAC canarypox virus.

2. A virus which is ALVAC.

3. A vector which comprises the virus of claim 1.

5. A virus as claimed in claim 2 further comprising exogenous DNA from a non-poxvirus source in a nonessential region of the virus genome.

6. A virus as claimed in claim 5 wherein the exogenous DNA is selected from the group consisting of rabies virus, Hepatitis B virus, Japanese encephalitis virus, yellow fever virus, **Dengue** virus, measles virus, pseudorabies virus, Epstein-Barr virus, herpes simplex virus, human immunodeficiency virus, simian immunodeficiency virus, equine herpes virus, bovine herpes virus, bovine viral diarrhea virus, human cytomegalovirus, canine parvovirus, equine influenza virus, feline leukemia virus, feline herpes virus, Hantaan virus, C. tetani, avian influenza virus, mumps virus and Newcastle Disease virus.

7. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is rabies virus, and the canarypox virus is vCP65 or vCP136.

8. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is human immunodeficiency virus and the canarypox virus is vCP95, vCP112, vCP60, vCP61, vCP125, vCP124, vCP126, vCP144, vCP120, vCP138, vCP117, vCP130, vCP152, vCP155, vCP156, vCP146, vCP148, vCP154, vCP168 or vCP153.

9. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is equine herpes virus and the canarypox virus is vCP132.

10. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is human cytomegalovirus and the canarypox virus is vCP139.

11. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is canine parvovirus and the canarypox virus is vCP123 or vCP136.

12. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Epstein-Barr virus and the canarypox virus is vCP167.

13. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is equine influenza virus and the canarypox virus is vCP128 or vCP159.

14. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is feline leukemia virus and the canarypox virus is vCP177, vCP83, vCP35, vCP37, vCP87, vCP93 or vCP97.

15. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is feline herpes virus and the canarypox virus is vCP162.

16. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Hantaan virus and the canarypox virus is vCP114 or vCP119.

17. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Hepatitis B and the canarypox virus is vCP169 or vCP157.

18. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is C. tetani and the canarypox virus is vCP161.

19. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is mumps virus and the canarypox virus is vCP171.

20. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is Japanese encephalitis virus and the canarypox virus is vCP107 or vCP140.

21. A virus as claimed in claim 6 wherein the virus is a canarypox virus, the non-poxvirus source is simian immunodeficiency virus, and the canarypox virus is vCP172.

22. A virus as claimed in claim 6 which is a rabies virus recombinant canarypox virus which is vCP65.

23. A virus as claimed in claim 6 which is a human immunodeficiency virus recombinant canarypox virus which is vCP95, vCP112, vCP60 or vCP61.

24. A virus as claimed in claim 1 further comprising exogenous DNA from a non-poxvirus source in a non-essential region of the virus genome.

25. An immunological composition for inducing an immunological response in a host animal inoculated with said composition, said composition comprising the virus of any one of claims 1, 2 or 5 to 24, or, a vector as claimed in claim 3 or 4, and a carrier.

26. The immunological composition of claim 25 which is a **vaccine**.

27. A method of expressing a gene product in a cell cultured in vitro comprising introducing into the cell a virus as claimed in any one of claims 1, 2 or 5 to 24, or, a vector, transforming cell with the expression vector, cultivating the transformed cell under conditions which allow expression of the recombinant poxvirus, and further purifying the protein as claimed in claim 3 or 4.

L32 ANSWER 12 OF 14 USPATFULL on STN
96:16887 NYVAC **vaccinia** virus recombinants comprising heterologous inserts.
Paoletti, Enzo, Delmar, NY, United States
Perkus, Marion E., Altamont, NY, United States
Taylor, Jill, Albany, NY, United States
Tartaglia, James, Schenectady, NY, United States
Norton, Elizabeth K., Latham, NY, United States
Riviere, Michel, Ecully, France
de Taisne, Charles, Lyons, France
Limbach, Keith J., Troy, NY, United States
Johnson, Gerard P., Waterford, NY, United States
Pincus, Steven E., East Greenbush, NY, United States
Cox, William I., Troy, NY, United States
Audonnet, Jean-Christophe F., Albany, NY, United States
Gettig, Russell R., Averill Park, NY, United States
Virogenetics Corporation, Troy, NY, United States (U.S. corporation)
US 5494807 19960227
APPLICATION: US 1993-105483 19930812 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A recombinant **vaccinia** virus wherein regions C7L.-K1L, J2R, B13R+B14R, A26L, A56R and I4L have been deleted therefrom, and further comprising exogenous coding DNA from a non-**vaccinia** source in a nonessential region of the **vaccinia** genome.

2. A recombinant **vaccinia** virus wherein the open reading frames for the thymidine kinase gene, the hemorrhagic region, the A type inclusion body region, the hemagglutinin gene, the host range gene region, and, the large subunit, ribonucleotide reductase have been deleted therefrom, and further comprising exogenous coding DNA from a non-**vaccinia** source in a nonessential region of the **vaccinia** genome.

3. A recombinant **vaccinia** virus as claimed in claim 2 wherein the non-**vaccinia** source is selected from the group consisting of rabies virus, Hepatitis B virus, yellow fever virus, **Dengue** virus, pseudorabies virus, Epstein-Barr virus, herpes simplex virus, simian immunodeficiency virus, equine herpes virus, bovine herpes virus, bovine viral diarrhea virus, human cytomegalovirus, canine parvovirus, equine influenza virus, feline leukemia virus, feline herpes virus, Hantaan virus, C. tetani, avian influenza virus, mumps virus and Newcastle Disease virus.

4. A recombinant **vaccinia** virus as claimed in claim 3, wherein the non-**vaccinia** source is rabies virus and the recombinant **vaccinia** virus is vP879 or vP999.

5. A recombinant **vaccinia** virus as claimed in claim 3, wherein the non-**vaccinia** source is Hepatitis B virus and the recombinant **vaccinia** virus is vP856, vP896, vP897, vP858, vP891, vP932, vP975, vP930, vP919, vP941 or vP944.

6. A recombinant **vaccinia** virus as claimed in claim 3, wherein the non-**vaccinia** source is yellow fever virus and the recombinant **vaccinia** virus is vP766, vP764, vP869, vP729, vP725, vP997, or vP984.

7. A recombinant **vaccinia** virus as claimed in claim 3, wherein the non-**vaccinia** source is **Dengue** virus and the recombinant **vaccinia** virus is vP867, vP962 or vP955.

8. A recombinant **vaccinia** virus as claimed in claim 3, wherein the non-**vaccinia** source is pseudorabies virus and the recombinant **vaccinia** virus is vP881, vP883, vP900, vP912, vP925, vP915 or vP916.

non-**vaccinia** source is Epstein-Barr virus and the recombinant **vaccinia** virus is vP941 or vP944.

10. A recombinant **vaccinia** virus as claimed in claim 3, wherein the non-**vaccinia** source is herpes simplex virus and the recombinant **vaccinia** virus is vP914.

11. A recombinant **vaccinia** virus as claimed in claim 3, wherein the non-**vaccinia** source is simian immunodeficiency virus and the recombinant **vaccinia** virus is vP873, vP948, vP943, vP942, vP952, vP948, vP1042, vP1071, vP943, vP942, vP952 or vP1050.

12. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is equine herpes virus and the recombinant **vaccinia** virus is vP1043, vP1025 or vP956.

13. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is bovine herpes virus and the recombinant **vaccinia** virus is vP1051, vP1074, vP1073, vP1083, vP1087 or vP1079.

14. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is bovine viral diarrhea virus and the recombinant **vaccinia** virus is vP972, vP1017 or vP1097.

15. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is human cytomegalovirus and the recombinant **vaccinia** virus is vP1001.

16. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is canine parvovirus and the recombinant **vaccinia** virus is vP998 or vP999.

17. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is equine influenza virus and the recombinant **vaccinia** virus is vP961 or vP1063.

18. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is feline leukemia virus and the recombinant **vaccinia** virus is vP1011.

19. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is Hantaan virus and the recombinant **vaccinia** virus is vp882, vP950 or vP951.

20. A recombinant **vaccinia** virus as claimed in claim 3 wherein the non-**vaccinia** source is C. tetani and the recombinant **vaccinia** virus is vp1075.

21. An immunological composition for inducing an immunological response in a host inoculated with the composition, said composition comprising a carrier and a recombinant virus as claimed in any one of claims 2, 33, 44, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

22. A method for expressing a gene product in a cell cultured in vitro, which method comprises introducing into the cell a modified recombinant virus as claimed in claim 2.

L32 ANSWER 13 OF 14 USPATFULL on STN
90:81596 Oxidized viruses or viral antigens and utilization for diagnostic prophylactic and/or therapeutic applications.
Quash, Gerard A., 12 Alle'des Charmilles, Franche Ville, France 69340
Rodwell, John D., 430 Ramsey Rd., Yardley, PA, United States 19067
McKearn, Thomas J., R.D. #3, Box 119, New Hope, PA, United States 18938
Ripoll, Jean P., 12 rue Freres Rizier, Cassieu, France
US 4965069 19901023
APPLICATION: US 1987-52518 19870520 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for protection of an animal or a human from an infection induced by a virus, comprising: administering to an animal or human an effective amount of a **vaccine** formulation which comprises a virus, viral antigen or fragment thereof having an oxidized oligosaccharide moiety which elicits a protective immune response, in which the oligosaccharide moiety was obtained by oxidation, by means of an agent selected from the group consisting of periodic acid, salts thereof, paraperiodic acid, salts thereof, metaperiodic acid, salts thereof and oxidase enzymes, of an oligosaccharide moiety of a virus, viral antigen or fragment thereof.

2. The method according to claim 1, in which the **vaccine** formulation

3. The method according to claim 1, in which the adjuvant is selected from the group consisting of: aluminum hydroxide, surface active substances, lysolecithin, pluronic polyols, polyanions and peptides.

4. The method according to claim 1 in which the infection is induced by a virus selected from the group consisting of herpesviridae, orthomyxoviridae, hepadnaviridae, parvoviridae, togaviridae, paramyxoviridae, flaviviridae, rhabdoviridae, bunyviridae, reoviridae picornaviridiae and retroviridae.

5. The method according to claim 1, in which the infection is induced by herpes simplex virus I or II.

6. The method according to claim 1, in which the infection is induced by cytomegalovirus.

7. The method according to claim 1, in which the infection is induced by hepatitis virus.

8. The method according to claim 1, in which the infection is induced by rubella virus.

9. The method according to claim 1, in which the infection is induced by measles virus.

10. The method according to claim 1, in which the infection is parainfluenza virus.

11. The method according to claim 1, in which the infection is **dengue** virus.

12. The method according to claim 1, in which the infection is human lymphoadenopathy-associated virus (LAV, HTLV-III, HIV).

13. The method according to claim 1, in which the infection is an auto-immune disease associated virus.

14. The method according to claim 1, in which the formulation is administered by an intradermal, intramuscular intraperitoneal, intravenous, or subcutaneous route.

L32 ANSWER 14 OF 14 USPATFULL on STN
89:62963 Carbohydrate perturbations of viruses or viral antigens and utilization for diagnostic prophylactic and/or therapeutic applications.
Quash, Gerard A., France Ville, France
Rodwell, John D., Yardley, PA, United States
McKearn, Thomas J., New Hope, PA, United States
Ripoll, Jean P., Chassieu, France
Cytogen Corporaton, Princeton, NJ, United States (U.S. corporation)
US 4853326 19890801
APPLICATION: US 1986-928631 19861118 (6)
PRIORITY: FR 1985-17377 19851125
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
CLM What is claimed is:
1. A method for detecting virus neutralizing antibodies in an aqueous sample, comprising: (a) contacting (i) a ligand which comprises a virus, viral antigen or fragment thereof, said virus, viral antigen or fragment thereof having perturbed oligosaccharide moiety, in which said perturbed oligosaccharide moiety was obtained by oxidation, by means of an agent selected from the group consisting of periodic acid, salts thereof, paraperiodic acid, salts thereof, metaperiodic acid, salts thereof, and oxidase enzymes, of an unperturbed oligosaccharide moiety, with (ii) an aqueous sample suspected of containing virus neutralizing antibodies in an assay system selected from the group consisting of an enzyme-linked immunosorbent assay, a radioimmunoassay, an agglutination assay, and an immunofluorescence assay; and (b) detecting any reaction with the ligand; in which any reaction with the ligand indicates the presence of neutralizing antibodies in the sample.

2. A method for detecting and quantitating virus neutralizing antibodies in an aqueous sample, comprising: (a) contacting (i) a ligand which comprises a virus, viral antigen or fragment thereof, said virus, viral antigen or fragment thereof having a perturbed oligosaccharide moiety, in which said perturbed oligosaccharide moiety was obtained by oxidation, by means of an agent selected from the group consisting of periodic acid, salts thereof, paraperiodic acid, salts thereof, metaperiodic acid, salts thereof, and oxidase enzymes, of an unperturbed oligosaccharide moiety, with (ii) an aqueous sample suspected of containing virus neutralizing antibodies in an assay system selected

radioimmunoassay, an agglutination assay, and an immunofluorescence assay; (b) detecting any reaction with the ligand; in which any reaction with the ligand indicates the presence of neutralizing antibodies in the sample; and (c) comparing any reaction of ligand and antibodies to that of a standard.

3. The method according to claim 1 or 2, in which the sample is an aliquot of a body fluid.

4. The method according to claim 3, in which the body fluid is serum or plasma.

5. The method according to claim 1 or 2, in which the aqueous sample is an aliquot of a solution of a monoclonal antibody.

6. The method according to claim 1 or 2, in which the virus neutralizing antibodies neutralize a virus selected from the group consisting of: adenoviridae, herpesviridae, orthomyxoviridae, hepadnaviridae, parvoviridae, togaviridae, paramyxoviridae, flaviviridae, rhabdoviridae, bunyviridae, reoviridae, picornaviridiae and retroviridae.

7. The method according to claim 1 or 2, in which the virus neutralizing antibodies neutralize cytomegalovirus.

8. The method according to claim 1 or 2 in which the virus neutralizing antibodies neutralize herpes simplex I or herpes simplex II virus.

9. The method according to claim 1 or 2, in which the virus neutralizing antibodies neutralize hepatitis virus.

10. The method according to claim 1 or 2, in which the virus neutralizing antibodies neutralize rubella virus.

11. The method according to claim 1 or 2, in which the virus neutralizing antibodies neutralize measles virus.

12. The method according to claim 1 or 2, in which the virus neutralizing antibodies neutralize parainfluenza virus.

13. The method according to claim 1 or 2, in which the virus neutralizing antibodies neutralize **dengue** virus.

14. The method according to claim 1 or 2, in which the virus neutralizing antibodies neutralize human lymphadenopathy-associated virus (LAV, HTLV-III, HIV).

15. The method according to clain: 1 or 2, further comprising the step of dissociating any immune complexes which may be present in the aqueous sample prior to contacting the ligand with the sample.

16. The method according to claim 1 or 2, in which the aqueous sample contains artially purified immunoglobulin.

17. A method for detecting virus neutralizing antibodies in an aqueous sample, comprising: (a) contacting a virus, viral antigen or fragment thereof with oxidizing agent selected from the group consisting of periodic acid, salts thereof, paraperiodic acid, salts thereof, metaperiodic acid, salts thereof, and oxidase enzymes to form a ligand which comprises a virus, viral antigen or fragment thereof, said virus, viral antigen or fragment thereof having a perturbed oligosaccharide moiety; (b) contacting the ligand with an aqueous sample suspected of containing virus neutralizing antibodies in an assay system selected from the group consisting of an enzyme-linked immunosorbent assay, a radioimmunoassay, an agglutination assay, and an immunofluorescence assay; (c) detecting any reaction with the ligand; in which any reaction with the ligand indicates the presence of neutralizing antibodies in the sample.

18. The method according to claim 17, which further comprises: (d) comparing any reaction of the ligand and antibodies to that of a standard in order to quantitate the virus neutralizing antibodies.

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(FILE 'HOME' ENTERED AT 11:02:05 ON 27 JUN 2006)

FILE 'USPATFULL' ENTERED AT 11:02:14 ON 27 JUN 2006
E ECKELS KENNETH/IN
L1 11 S E3-E4
E PUTNAK JOSEPH R/IN

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L3            0 S L1 NOT L1
                E DUBOIS DORIA R/IN
L4            6 S E3
L5            0 S L4 NOT L1

     FILE 'WPIDS' ENTERED AT 11:03:30 ON 27 JUN 2006
                E ECKELS K/IN
L6           13 S E3 OR E4
                E PUTNAK J R/IN
L7           12 S E3
L8            3 S L7 NOT L6
                E DUBOIS D R/IN
L9           29 S E3
L10          20 S L9 NOT (L6 OR L8)
L11           1 S L10 AND (DEN? OR FLAVIVIR?)

     FILE 'MEDLINE' ENTERED AT 11:05:22 ON 27 JUN 2006
                E ECKELS K/AU
L12          64 S E3-E5
L13          50 S L12 AND (FLAVIVIR? OR DEN?)
L14          42 S L13 AND VACCIN?
L15           1 S L14 AND (MULTIVALENT)
L16          41 S L14 NOT L15
                E PUTNAK J R/AU
L17          21 S E3-E4
L18          17 S L17 NOT L16
L19          11 S L18 AND (FLAVIVIR? OR DENG?)
                E DUBOIS D R/AU
L20          31 S E3
L21           9 S L20 NOT (L12 OR L17)
L22        2919 S (DENGUE VIRUS)
L23          13 S L22 AND (45AZ5 OR S16803 OR CH53489 OR 341750)
L24           5 S L23 NOT (L12 OR L16)
L25         418 S L22 AND VACCIN?
L26           6 S L25 AND MULTIVALENT

     FILE 'USPATFULL' ENTERED AT 11:09:48 ON 27 JUN 2006
L27        2786 S (DENGUE)
L28        2091 S L27 AND VACCIN?
L29        1107 S L28 AND ATTENUATE?
L30         179 S L29 AND MULTIVALENT
L31          54 S L30 AND AY<2001
L32          14 S L31 AND DENGUE/CLM

=> log off
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:y
STN INTERNATIONAL LOGOFF AT 11:11:09 ON 27 JUN 2006
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